proteinScape 3.0
User Manual

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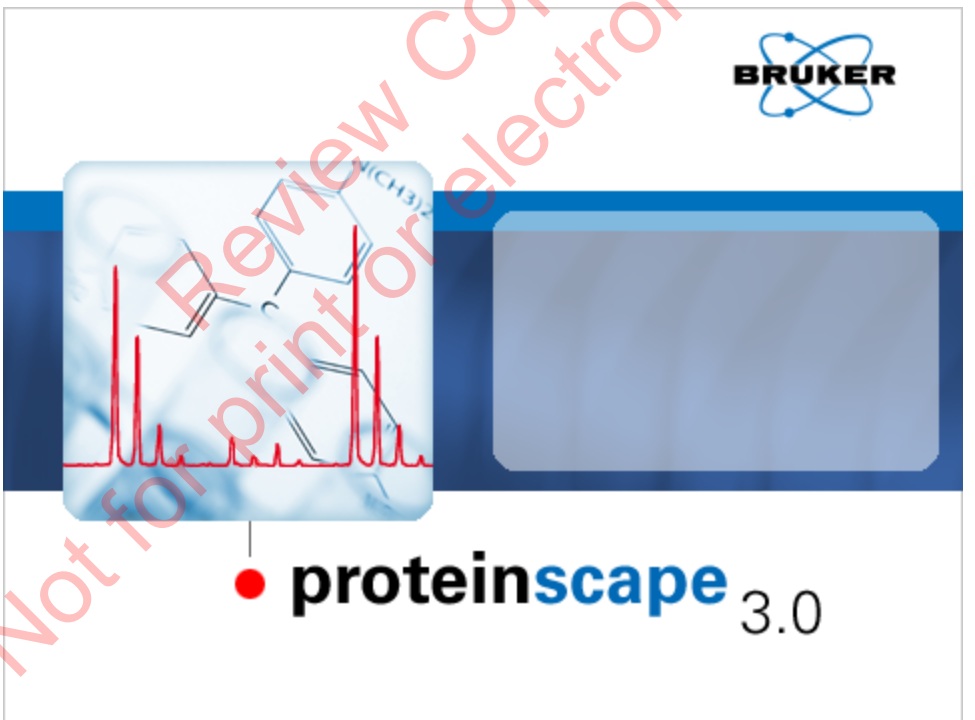

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Revision 1 (November 2011)

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Typographic and Application Conventions

These conventions list the font styles used for indicating specific graphical user interface (GUI) elements (for example, Menu Items).

They also list the meanings of terms used to describe user interactions with the graphical user interface of software (for example, right-click).

Typographic Conventions

File and Directory Names and Paths

File and directory names and paths are written in `monospace type`.
Example: "Navigate to the `C:\Bruker\solariXcontrol\test` directory and open the file `test_1.txt`.

GUI elements

GUI (graphic user interface) elements are options that are available in software that enable a user to interact with the software without using the keyboard.
Typical GUI elements include windows, menus, menu options, drop-down lists, and buttons.

- Names of windows are written in regular, capitalized type.
- Names of text entry fields and buttons are written in **bold type**.

  Example: "In the New Digest window, enter values for **Name** (obligatory) and **Note** (optional) and click **Next >**.

- GUI menus and options are written in **bold type**.

  Example: "Select **Window > Show View > LC-MS Survey**".

Keyboard

References to keys on the keyboard are written in **bold type**.
Example: "Press **ALT+F4**".

Publications

References to electronic and printed documents are written in *italic type*.
Example: "See the *ProteinScape Administrator Manual* for more details".

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URLs

Uniform Resource Locators (URLs) written in regular, underlined type. The scheme name (for example, http://) is omitted.
Example: "See the pages at www.mascot.org/users for more details".

GUI User Interactions

Clear

Deactivate a software feature by positioning the mouse pointer over a selected check box and pressing the left mouse button.
Example: "Clear the **Curve Smoothing** option."

Click

Position the mouse pointer over a GUI element and press the left mouse button to start an action in the software.
Example: "Click **OK**."

Click and drag

Position the mouse pointer in a window and press the left mouse button. Hold the left mouse button down and move the mouse pointer to select a (usually rectangular) area.
Example: "Zoom in on peaks of interest by clicking and dragging in the Spectrum window."

Double-click

Position the mouse pointer over a GUI element and press the left mouse button twice in quick succession to start an action in the software.
Example: "Double-click the Sample symbol."

Drag and drop

Position the mouse pointer over a GUI element and press the left mouse button. Hold the left mouse button down and move the selected element to a new location.
Example: "Drag and drop the tab into the desired pane."

Select

Navigate to a desired menu command.
Example: "Select **File > Zip > Zip Project…**"

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Position the mouse pointer over a GUI element (for example, a table entry) and press the left mouse button to highlight it.
Example: "Select the desired file in the list and click **Export**."
Activate a software feature by positioning the mouse pointer over an empty check box and pressing the left mouse button.
Example: "Select the **Curve Smoothing** option."

Right-click

Position the mouse pointer over a GUI element and press the right mouse button to open a menu for the selected element.
Example: "Right-click in the **Spectrum View** window".

Keyboard Conventions

Press

Use the indicated key(s) on the keyboard. When modifier keys (for example, **SHIFT**, **ALT**, **CTRL**) are indicated, keep them pressed while pressing the other key.
Example: "To switch between table and **Spectrum View** press **CTRL+F3**."

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ProteinScape — the Bioinformatics Platform

ProteinScape is Bruker Daltonics' central bioinformatics platform, developed to provide researchers with sophisticated tools for the analysis and evaluation of proteomic data. It supports various gel- and LC-based workflows. The embedded processing pipeline uses powerful algorithms to optimize the outcome and maximize the reliability of your protein identification.

The ProteinScape database efficiently organizes data — for example, LC-data, gel data, mass spectra, process parameters, search results, and data evaluation — for all types of proteomics projects. By doing so, ProteinScape acts as a central control unit and data evaluation tool for mass-spectrometry based protein identification, characterization, and quantitation.

Identifying Proteins

ProteinScape identifies proteins on the basis of peptide mass spectra. Peptide mass spectra from protein digests are analyzed, assigned a sequence, and protein databases are searched for the presence of the peptide sequences.

The higher the scores of the peptide sequences identified in the candidate protein, the higher the confidence in the identification.

Identifying Glycans

ProteinScape is also able to identify glycan moieties in glycoproteins. Mass spectra are analyzed for the presence of characteristic patterns and fragments that correspond to entries in glycan databases.

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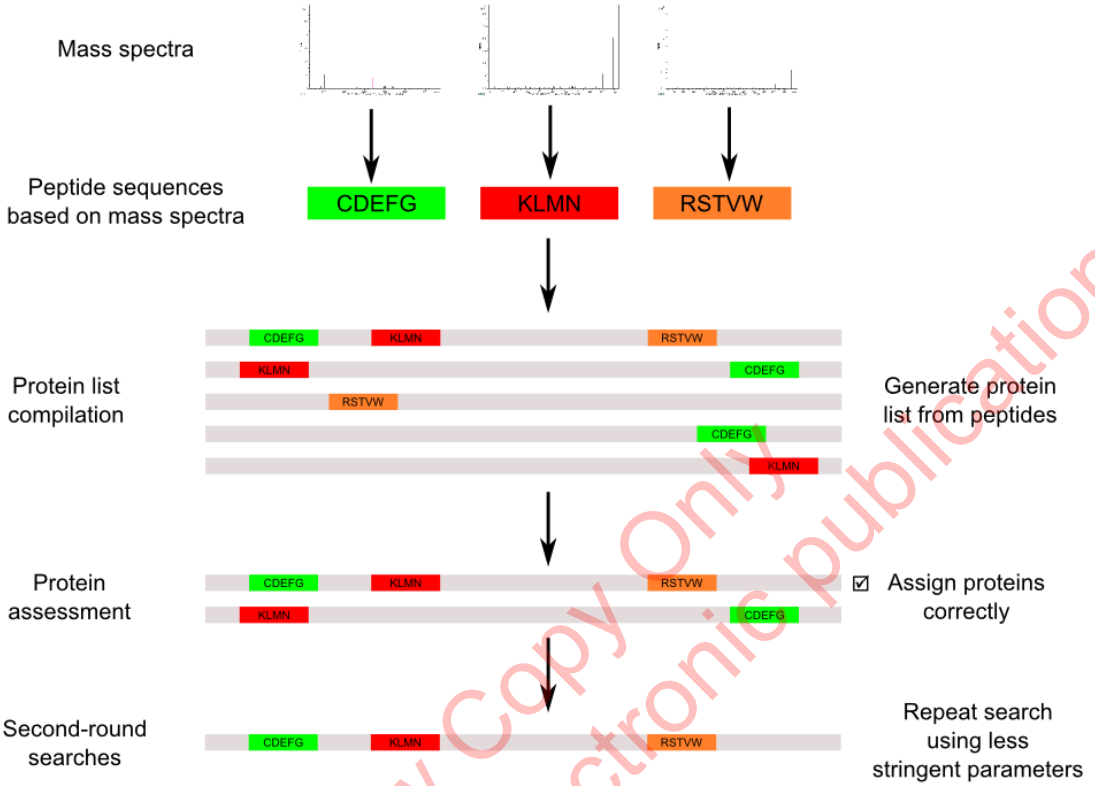

Identifying proteins using ProteinScape

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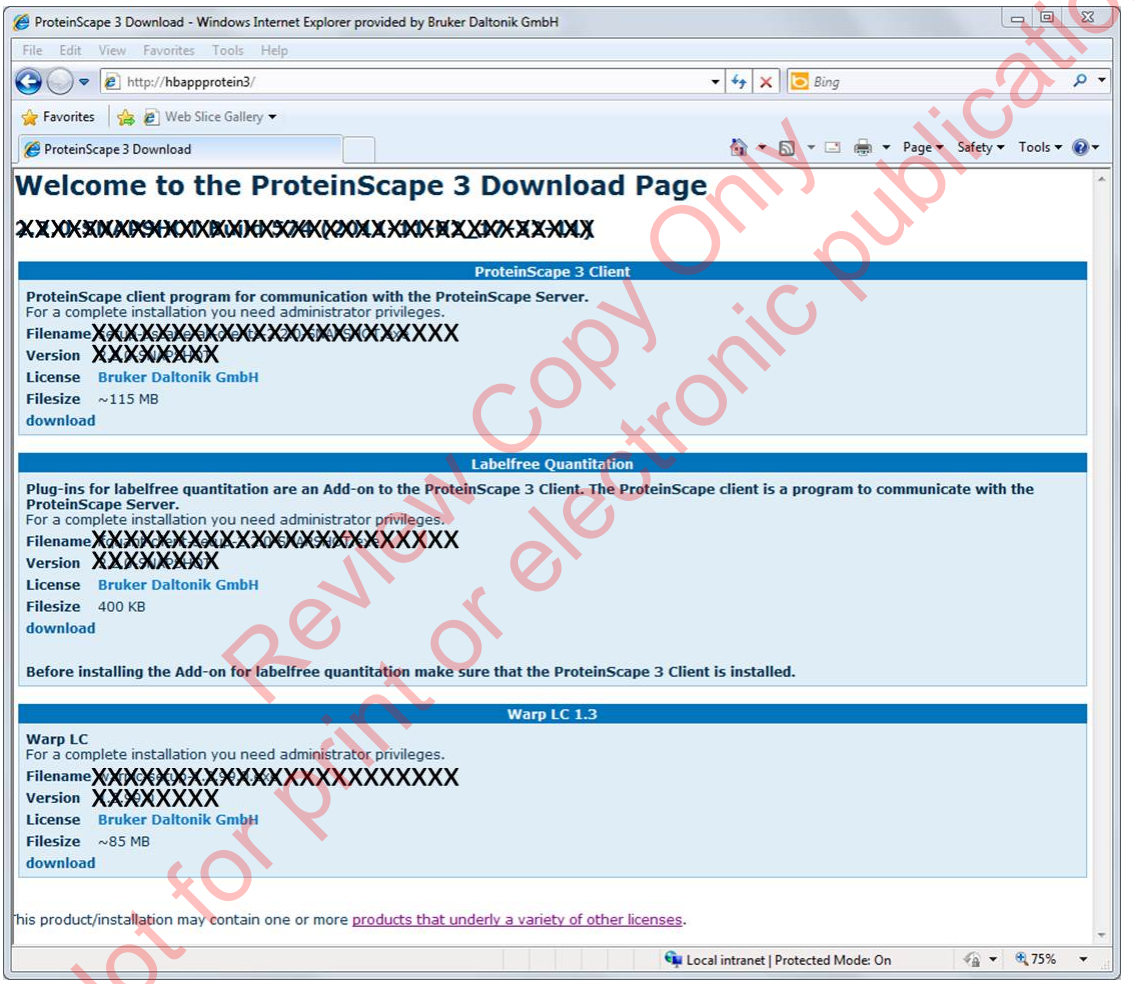
1 Installing the ProteinScape Client

ProteinScape is installed on a server and is accessed through a client program that must be installed on the user's PC. The client program can be downloaded from the ProteinScape server using an Internet browser. Type the address *http:\\your_server_URL* into the Internet browser's address bar to go to the download page (see Figure 1-1). Click **download** to start installation of the client (Administrator privileges are required).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ProteinScape 3 Client
ProteinScape client program for communication with the ProteinScape Server.
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For a complete installation you need administrator privileges.
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Version
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Before installing the Add-on for labelfree quantitation make sure that the ProteinScape 3 Client is installed.
Warp LC 1.3
Warp LC
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Figure 1-1 Download the client program from the ProteinScape server

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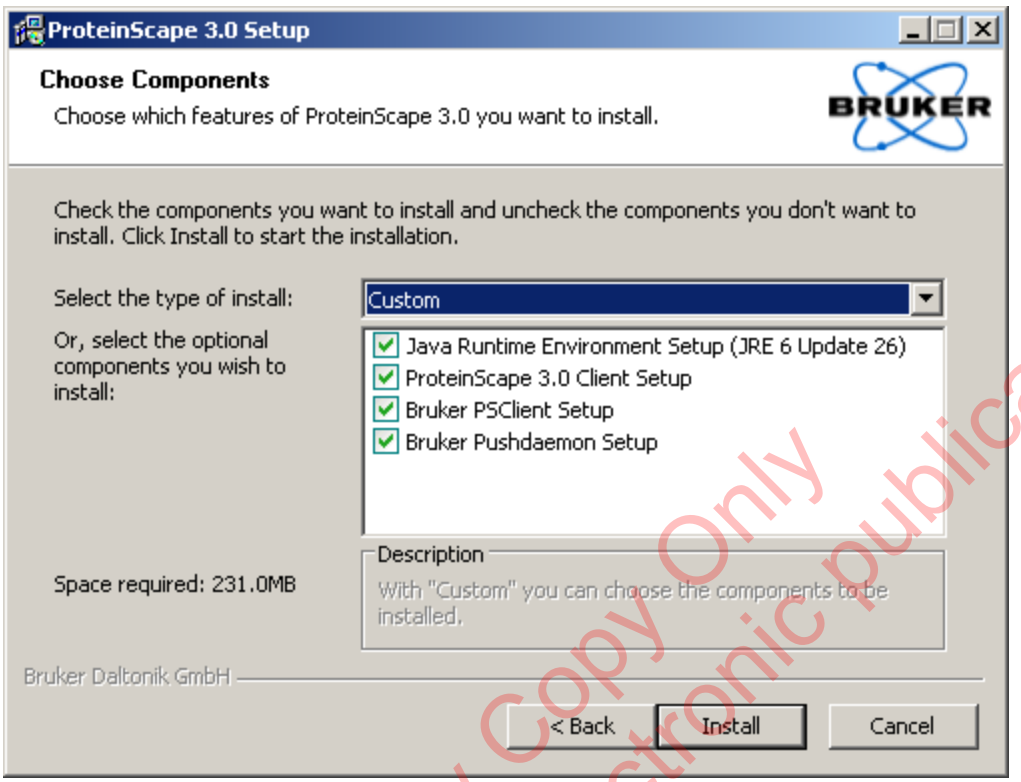
ProteinScape 3.0 Setup
Choose Components
Choose which features of ProteinScape 3.0 you want to install.
BRUKER
Check the components you want to install and uncheck the components you don't want to install. Click Install to start the installation.
Select the type of install:
Custom
Or, select the optional components you wish to install:
Java Runtime Environment Setup (JRE 6 Update 26)
ProteinScape 3.0 Client Setup
Bruker PSClient Setup
Bruker Pushdaemon Setup
Description
With "Custom" you can choose the components to be installed.
Space required: 231.0MB
Bruker Daltonik GmbH
< Back
Install
Cancel

Figure 1-2 Custom installation of ProteinScape with choice of software to be installed

During the installation, the user can choose which components are installed. We recommend selecting the **Complete** option from the drop-down menu during setup. This option installs:

1. The Java Runtime Environment, if necessary
2. The ProteinScape 3.0 Client
3. The PSClient module, required for workstations running BioTools or WARP-LC) and
4. The Push Daemon, required for ESI instruments running HyStar (see section 4.1.2.1).

Selecting the **Custom** option enables deselection of standard setup components (see Figure 1-2).

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1.1 User Settings

After opening ProteinScape for the first time, all ProteinScape settings are stored on the client's hard disk.

The default location is: `C:\documents and settings\Windows User name\Application data\Bruker Daltonik\ Server Access\workspaces\PS user@server name.`

Under Windows 7 the default location is: `C:\Users\Windows user name\AppData\Roaming\Bruker Daltonik\Server Access\workspaces.`

In this way, the personal settings, perspectives etc. are saved for each user/server combination.

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2 The ProteinScape Client Workspace

The default perspective of the ProteinScape client workspace (see Figure 2-1) contains four panes:

- an upper-left pane displaying the **Project Navigator** tree
- an upper-right pane containing the **Main View** displaying detailed information on the selected **Project Navigator** node
- two lower panes displaying graphical and tabular Views.

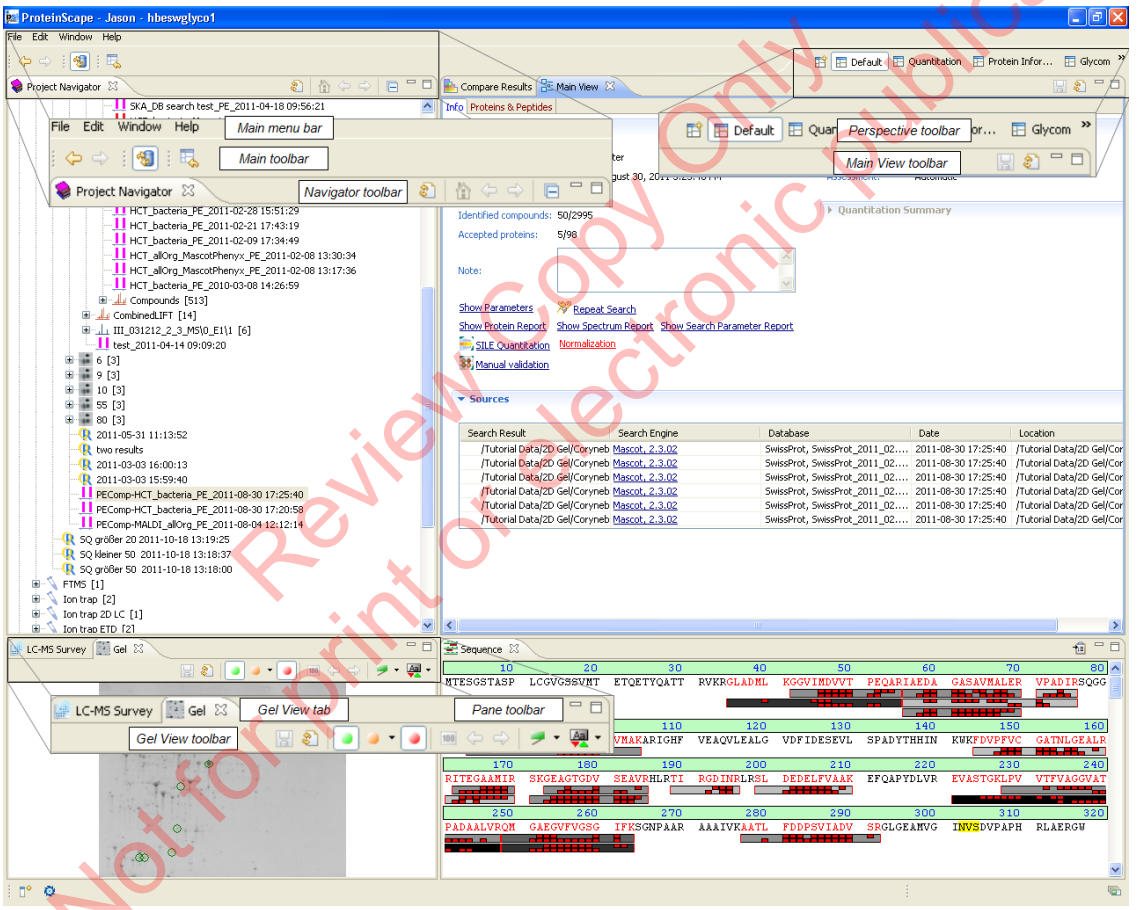

Figure 2-1 ProteinScape client workspace

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2.1 Organizing Panes and Views

Each pane contains one or more Views. Views in the background can be brought to the foreground by clicking their tab.

Clicking the “X” on a View’s tab closes the View.

Note If a pane contains only one View, closing the View will also close the pane.

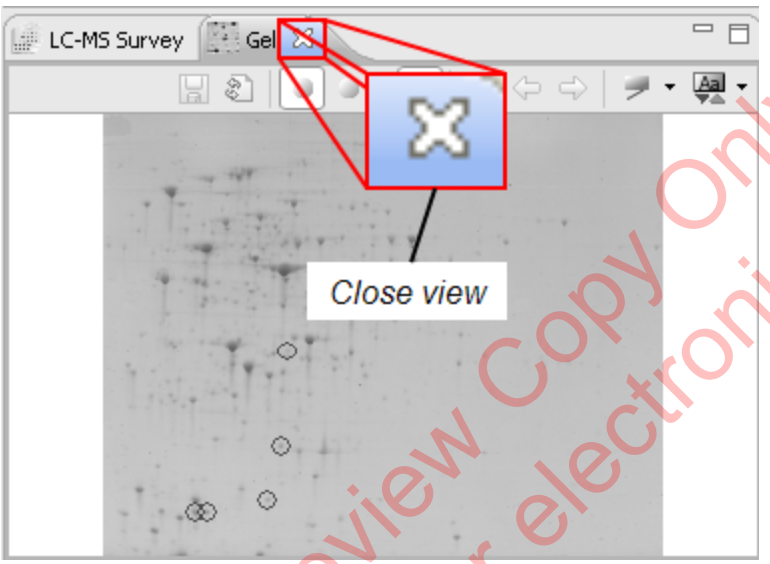

Figure 2-2 **A pane containing the Gel View (foreground) and LC-MS Survey View (background)**

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2.1.1 View Tab Shortcut Menu

Each View contains a tab with a symbol and the View's label. Right-clicking a View's tab opens a shortcut menu containing commands used to control the location and appearance of the View (see Table 2-1). This shortcut menu is identical in every View.

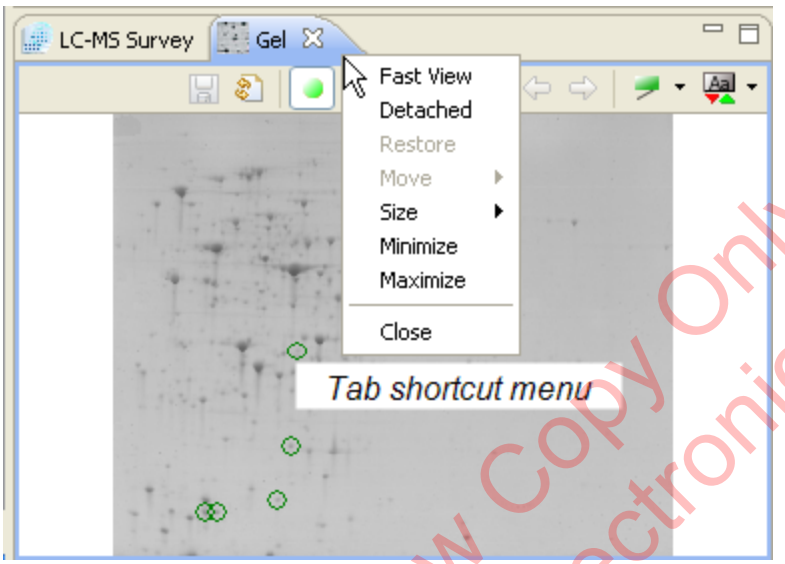

Figure 2-3 View shortcut menu

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Table 2-1 View shortcut menu commands

Shortcut menu command	Action
Fast View	Moves the View to the Fast View region (see section 2.1.4).
Detached	Detaches the chosen View from the workspace. The View remains in the foreground and can be moved freely, even outside the ProteinScape workspace (for example, to a second screen in dual-screen setups). Reattach the View to the workspace by clearing the **Detached** option in the shortcut menu.
Restore	Restore the View to its original position.
Move	Allows the View or pane to be moved to another location in the workspace.
Size	Select the desired border (Left, Right, Top or Bottom) and use the respective arrow key to move the edge of the View.
Minimize	Collapse the pane to an symbol toolbar at the edge of the screen. Reset the perspective View using the Restore symbol (see section 2.1.2).
Maximize	Expand the pane to full-screen View. Reset the perspective using Restore.
Close	Close the respective View.

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2.1.2 Expanding and Collapsing Panes

Panes can be expanded to fill the workspace by clicking the **Maximize** symbol in the pane toolbar or selecting the **Maximize** command in a View's shortcut menu (see section 2.1.1).

Panes can be collapsed to the edge of the workspace by clicking the **Minimize** symbol in the pane toolbar or selecting the **Minimize** command in a View's shortcut menu (see section 2.1.1).

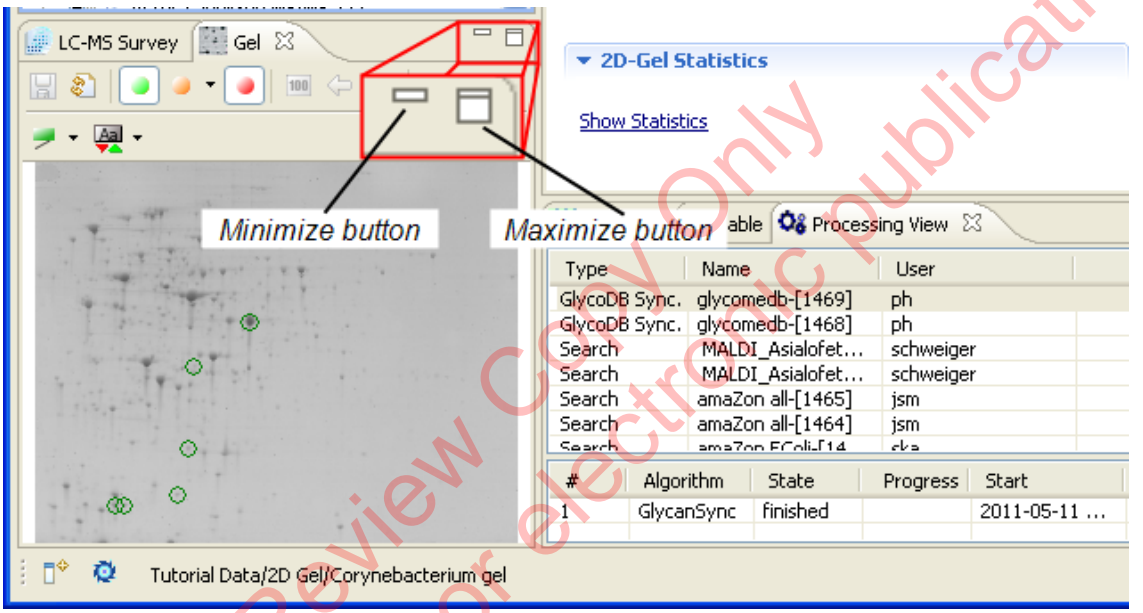

Figure 2-4 The Maximize button expands a pane to fill the workspace; the Minimize button collapses the pane to the edge of the workspace.

Click the **Restore** button in the View toolbar to reset the perspective after maximizing a pane.

Click the **Restore** button in the lower right corner of the workspace to reset the perspective after minimizing a pane.

2.1.3 Changing the Size of Panes

The relative height and width of panes can be changed by placing the pointer over a pane's boundary and dragging the pointer in the appropriate direction (Figure 2-5).

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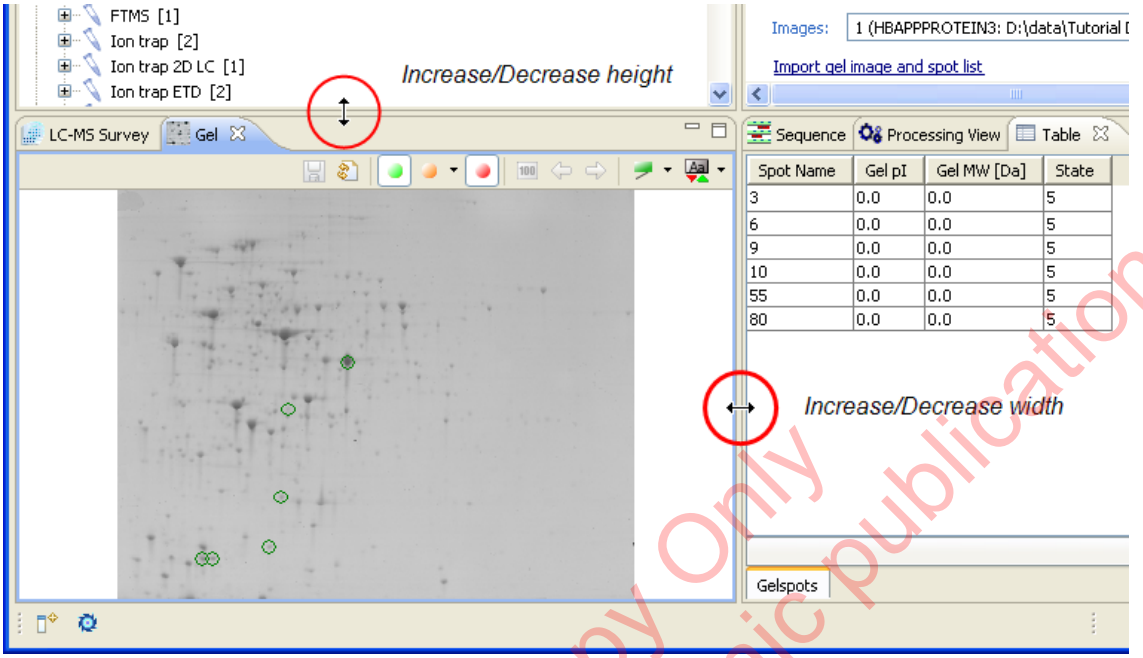

Figure 2-5 Changing the height and width of panes

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2.1.4 Using Fast View

Selecting the **Fast View** command from the View shortcut menu sends the View to the Fast View region.

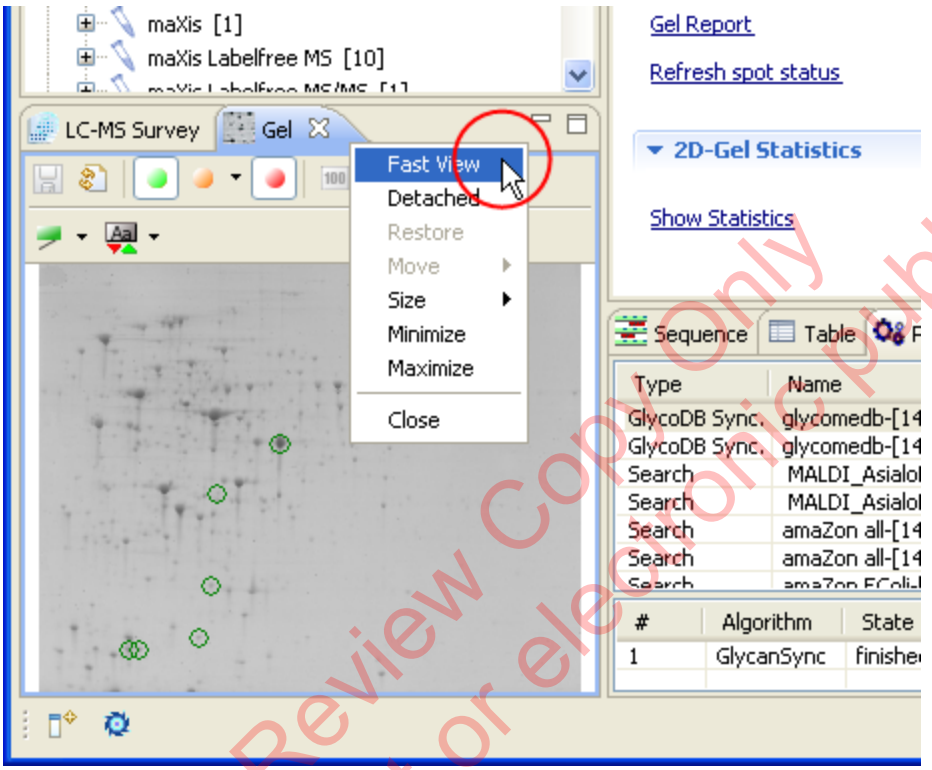

Figure 2-6 Sending a View to the Fast View region

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The Fast View region is a group of symbols in the lower-left corner of the workspace.

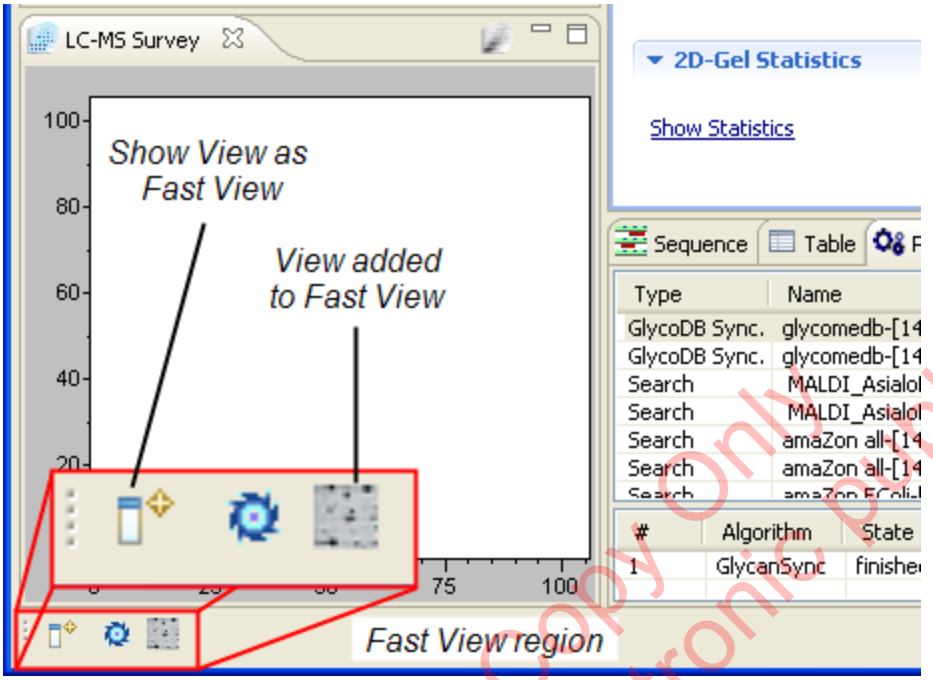

Figure 2-7 The Fast View region in the lower-left corner of the workspace

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Clicking a View's symbol in the **Fast View** region temporarily expands the View.

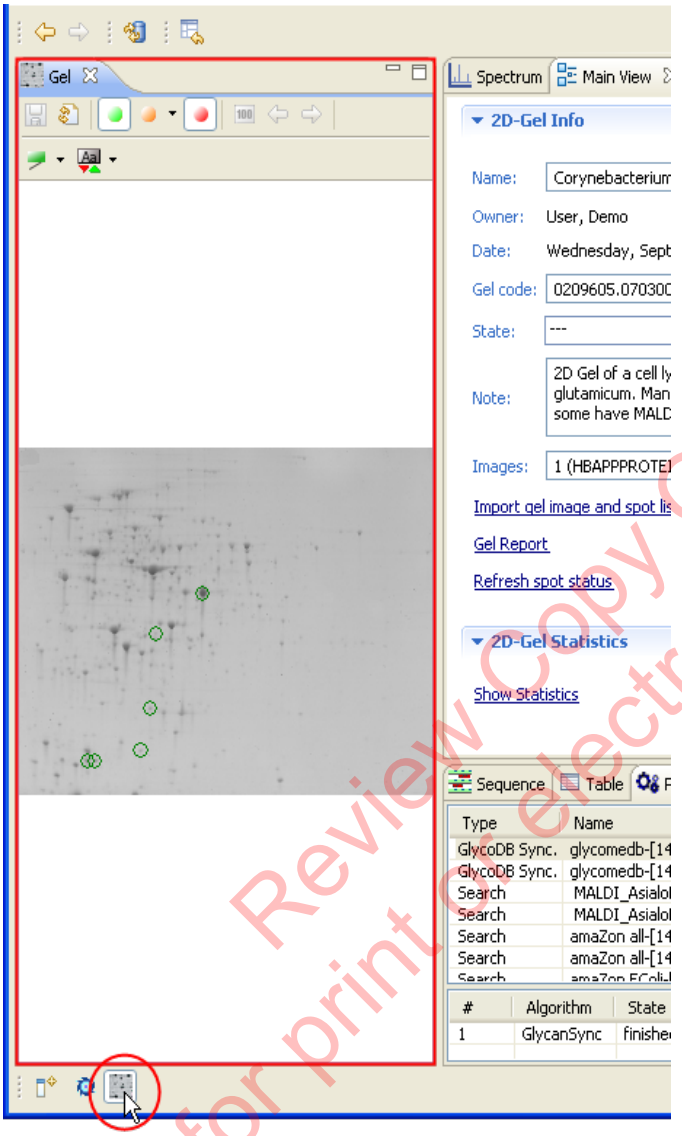

Figure 2-8 Temporarily expanding a View in the Fast View region

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The **Orientation** (**Horizontal** [= landscape] or **Vertical** [= portrait]) of the temporary View can be selected in the shortcut menu of the symbol in the Fast View region.

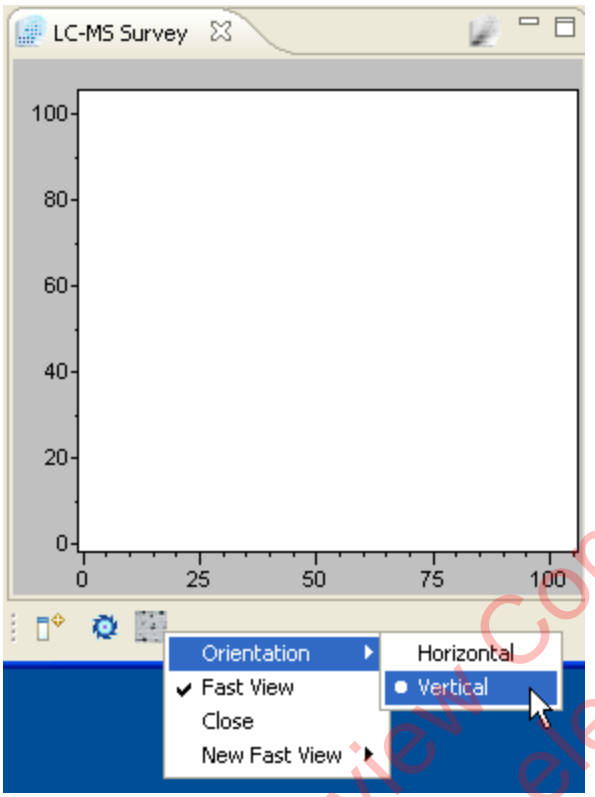

Figure 2-9 Setting the orientation of the temporarily expanded View

If the user clicks the View's symbol again or clicks anywhere else in the workspace, the View collapses back into the Fast View region.

To return the View to its original pane, clear the **Fast View** option in the symbol's shortcut menu.

Select **Close** to close the View.

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To add an additional View to the Fast View region, either:

- Select a View using **New Fast View** in the View symbol shortcut menu

or

- Click the **Show View as Fast View** button and select a View from the list.

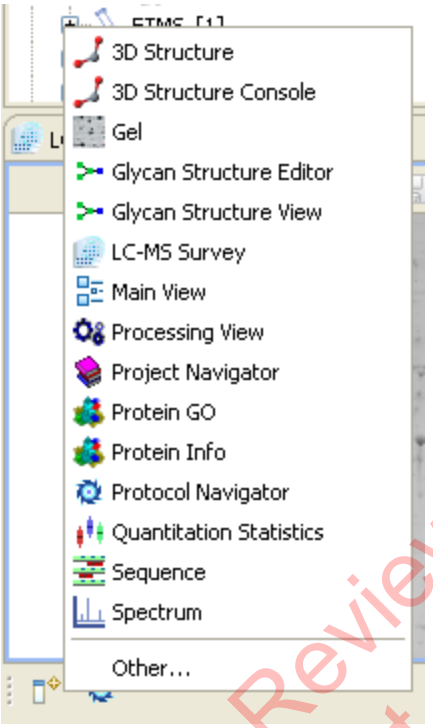

Figure 2-10 Adding a View to the Fast View region using the Show View as Fast View button

2.1.5 Moving Views and Panes within the Workspace

A View can be moved into a different pane by dragging and dropping its tab into the pane or pane toolbar (see Figure 2-11). The gray frame indicates where the View or tab will be located after moving.

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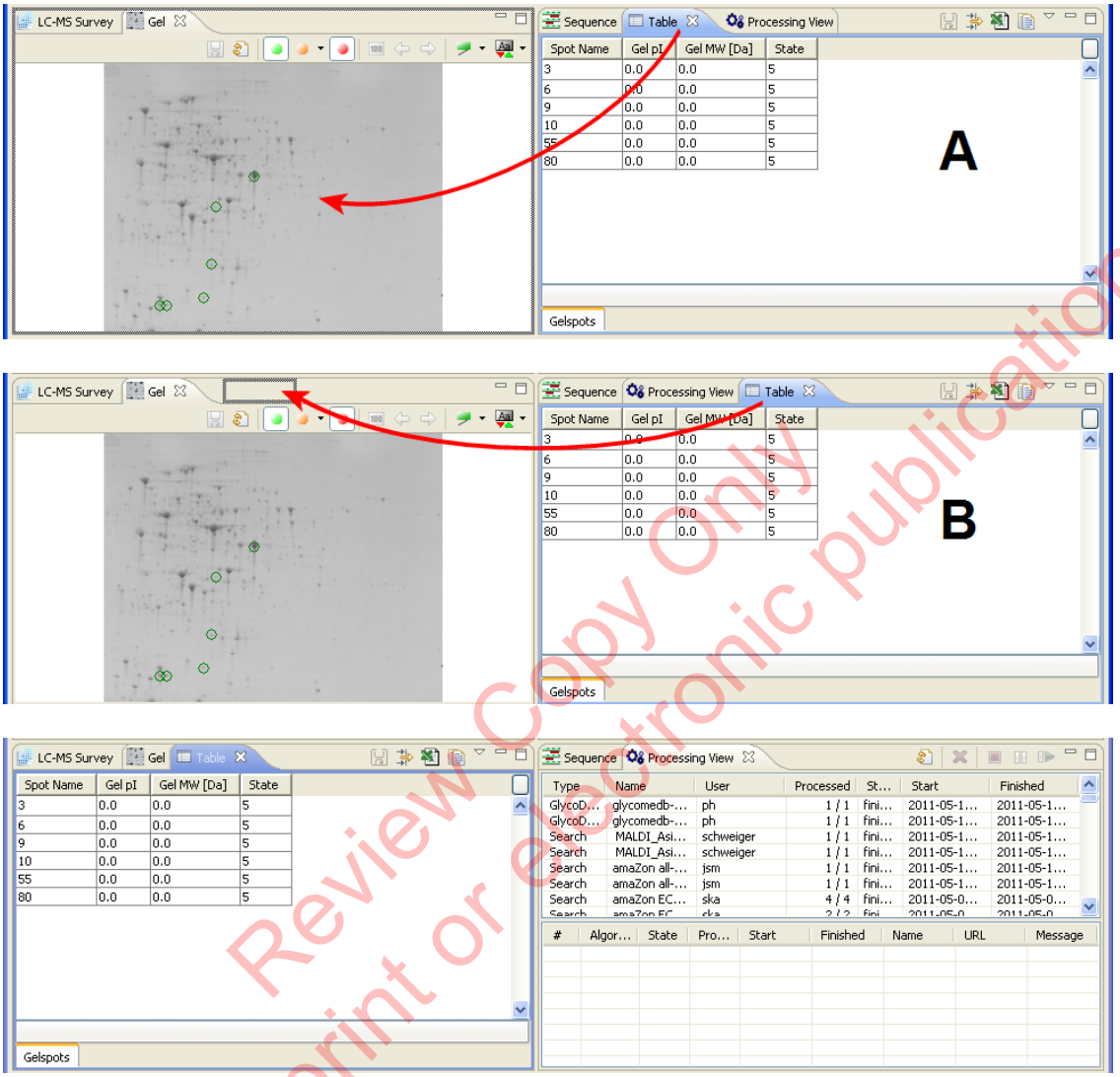

Figure 2-11 Moving a View into a different pane

A new pane is created by dragging and dropping the tab of a View over another pane's border (see Figure 2-12). The gray frame indicates where the new pane will be located.

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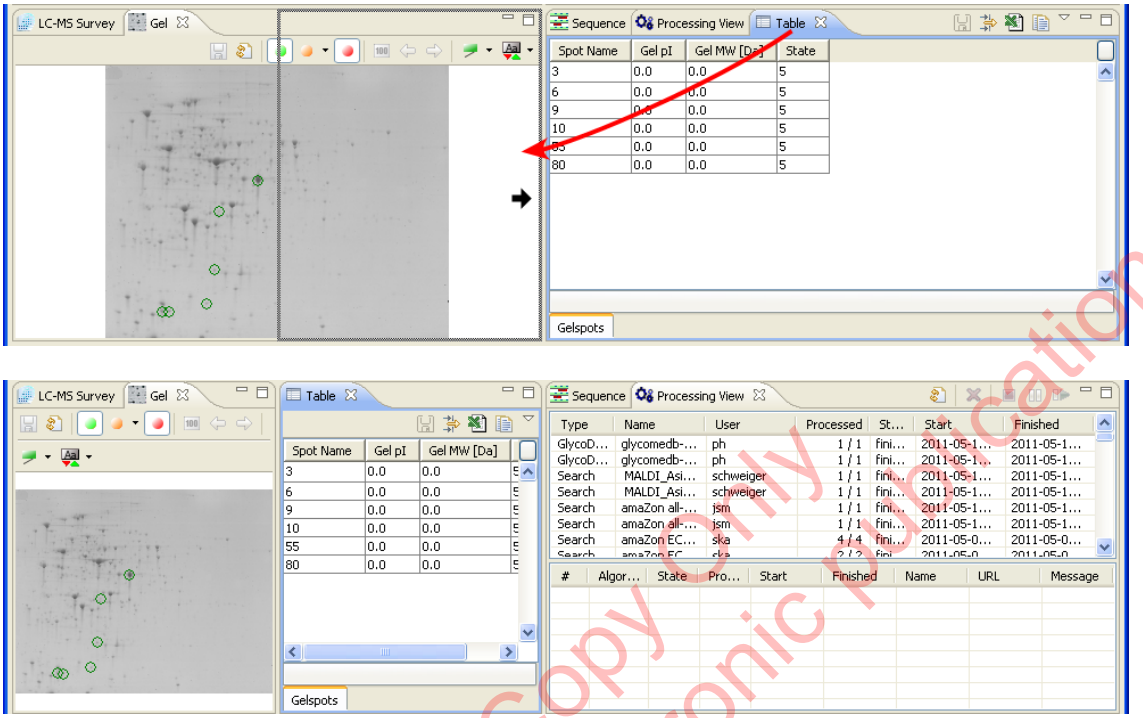

Figure 2-12 **Dragging and dropping a View over another pane's border to create a new pane**

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Alternatively, a View or pane can be moved using the **Move** command in the View shortcut menu.

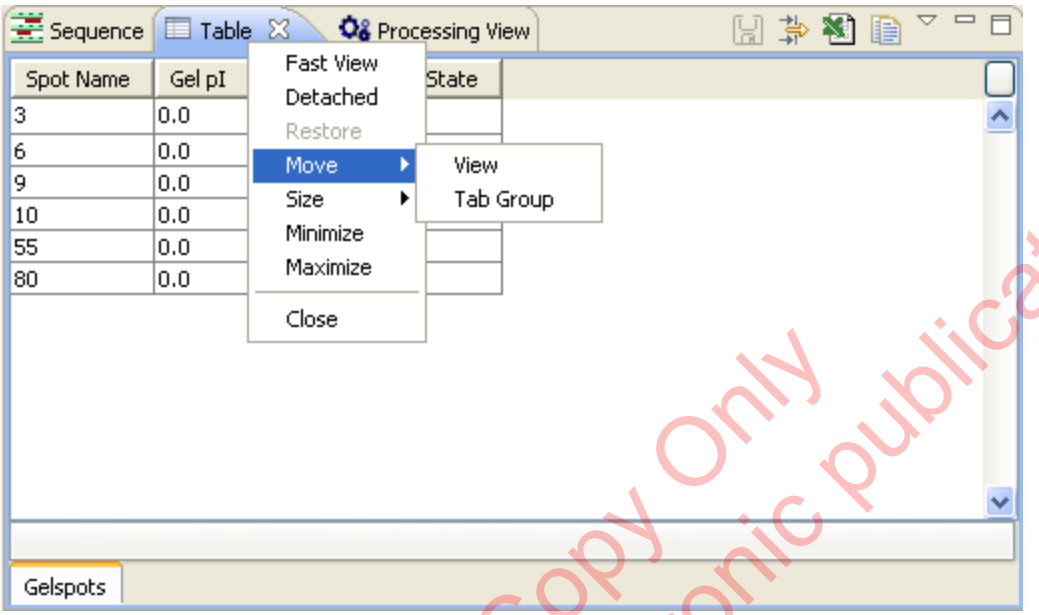

Figure 2-13 The Move command in the View shortcut menu is used to move Views and panes in the workspace

Selecting **Move > View** or **Move > Tab Group** creates a gray frame that can be moved anywhere inside or outside the workspace. Move the gray frame to the desired location and click to finish the operation.

Views can be moved into existing panes, into a new pane, or outside the workspace (for example, to a second screen in dual-screen setups).

A pane (= **Tab Group**) can be moved into a new pane or outside the workspace.

2.2 Working with Perspectives

The arrangement of panes and Views in the workspace is called a perspective.

Perspectives are managed using the toolbar in the upper-right corner of the workspace.

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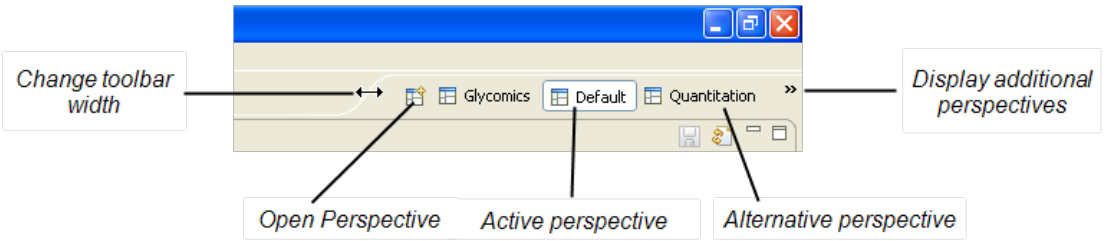

Figure 2-14 The perspectives toolbar

The active perspective is indicated by a white background. Switch between perspectives by:

- clicking another perspective button
- clicking the **Open Perspective** button and selecting **Other** to access all available perspectives through the **Open Perspective** window (see Figure 2-15).
- clicking the unfold button to display additional perspectives and selecting the desired perspective.

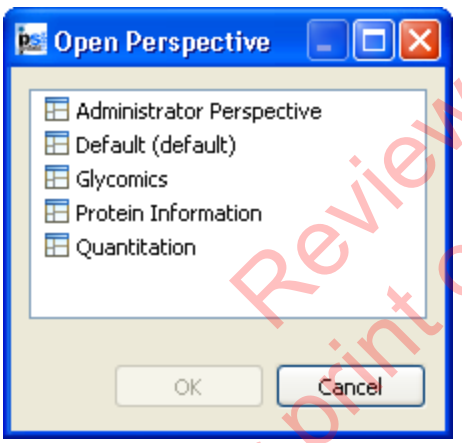

Figure 2-15 Accessing alternative perspectives through the Open Perspective window

The width of the toolbar and number of perspectives displayed can be changed by placing the mouse pointer over the left edge of the toolbar boundary and dragging the pointer to the left or right.

Additional commands are available in the active perspective shortcut menu.

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Table 2-2 Active perspective shortcut menu commands

Shortcut menu command	Action
Customize	Advanced customization features.
Reset	Resets the active perspective to the saved configuration. This is very useful if the perspective has been changed by mistake.
Close	Closes the perspective and hides the perspective button.
Dock on	Allows the perspectives menu bar to be relocated within the workspace.
Show Text	Show or hide perspective labels in the perspective tool-bar buttons.

Resetting a Perspective

If a View is accidentally closed or dragged to the wrong pane, select **Window > Reset Perspective** or click the **Reset Perspective** button ( ) in the main toolbar to restore the active perspective to the saved configuration.

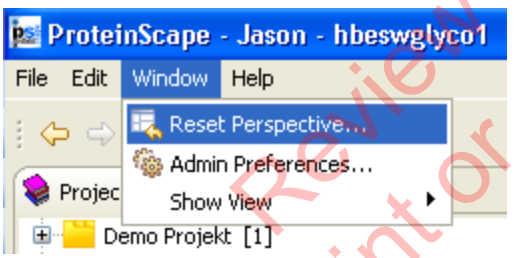

Figure 2-16 Resetting a perspective

Users can configure, save and load their own customized perspectives for specific tasks — for example, PMF analysis of gel spots or quantitation studies on a labeled LC-MS/MS run.

2.2.1 Creating Perspectives

New perspectives can be saved by right-clicking the active perspective button and selecting **Save As** in the shortcut menu.

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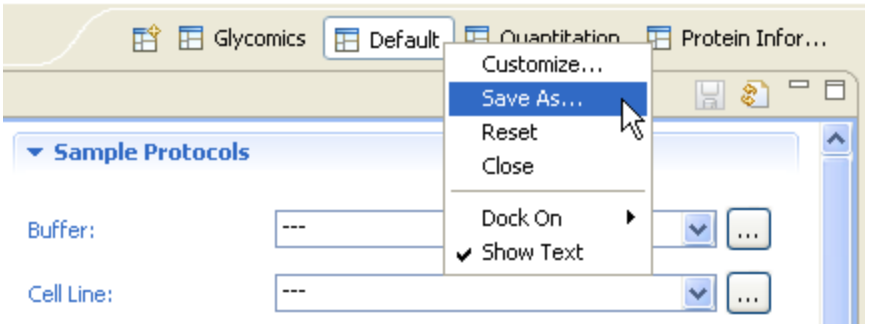

Figure 2-17 Select Save As to create a new perspective

The existing perspective can be overwritten, or a new perspective can be created by typing a new **Name** in the **Save Perspective As** dialog box.

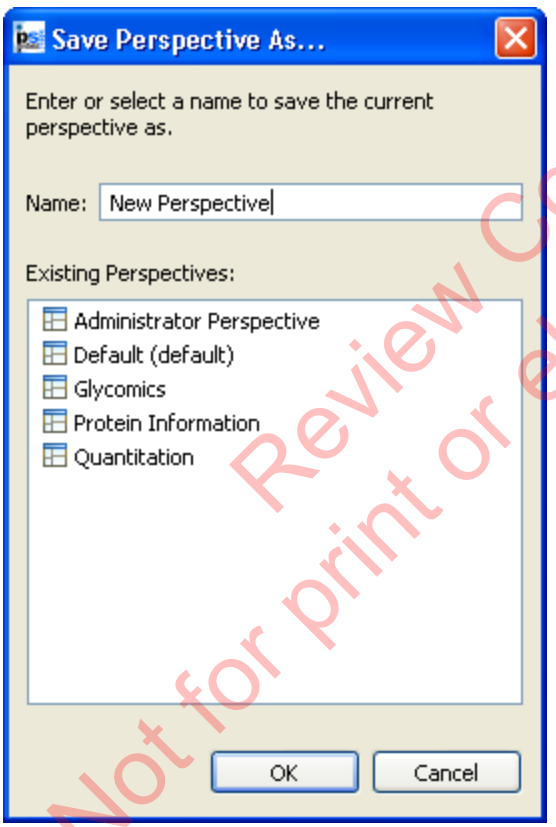

Figure 2-18 Save Perspective As dialog box

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2.3 Main Menu Bar — Menus and Commands

The main menu bar contains the following menus and commands:

File Menu

- **Logout** — Log out and log in as a different user.
- **Change Password** — Enables users to change their password.
- **Exit** — Exits ProteinScape.

Edit Menu

- **Methods > Protein/Glycan MS Search Methods** or **MS/MS Search Methods** — Opens the respective search parameter configuration in the **Protein/Glycan Searches** dialog box.
- **Classification** — Opens the **Spectra Classification Parameters** dialog box.

Window Menu

- **Reset Perspective** — Resets the active perspective to the saved configuration. This is very useful if the perspective has been re-arranged (for example, a View was closed by mistake).
- **Admin Preferences** — Opens the **Administrator Preferences** window. This command is enabled only if the user is a member of the administrator group. Details can be found in the *ProteinScape Administrator Manual*.
- **Show View** — All available Views are listed in this menu. They can be selected and activated in the current perspective. If a View was closed by mistake, it can always be found and re-opened here.

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Help Menu / F1

- **F1** opens the dynamic help in all contexts, and displays a brief description of the context and links to the relevant chapters in the extensive online help.
- **Help Contents** — Opens the online help. All chapters can be easily accessed from here.
- **Search** — A full text search of the entire online help.
- **Dynamic Help** — This is the context-sensitive help. It can also be started using the F1 key.
- **What's New?** — Information on the new features in ProteinScape.
- **Client Log** — Select a location to save a zip package of client log files. These files may be useful for Bruker support diagnostics.
- **About ProteinScape** — Information about the current program version.

2.4 Main Toolbar

The buttons in the main toolbar are used to perform actions in the **Project Navigator View** and to reset the active perspective.

Table 2-3 Main toolbar buttons

Button	Name	Action
	Back	Go back one step in a sequence of node selections.
	Forward	Go forward one step in a sequence of node selections.
	Auto Refresh	Switch on/off automatic updates of the **Project Navigator** tree.
	Reset Perspective	Resets the active perspective to the most recently saved configuration.

2.5 Main View

The **Main View** window is used to display information about the currently selected **Project Navigator** tree node.

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Main View information is displayed in one or more areas (see Appendix B). Click ▸ to expand or ▾ to collapse areas.

Table 2-4 Main View toolbar buttons

Button	Name	Action
	Save	Save information in **Main View** areas to the database.
	Refresh	Updates the page content by reloading data from the database.

2.6 Tabular View Shortcut Menu

There are a number of tabular views in ProteinScape. The shortcut menus of these tables contain some or all of the following features.

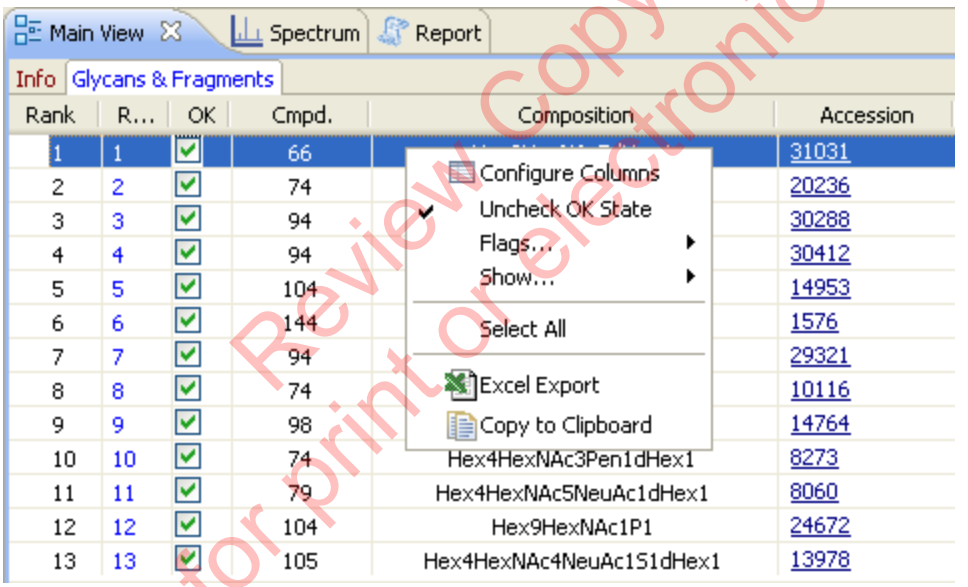

Figure 2-19 Example of a tabular View shortcut menu (Glycans & Fragments table)

Configuring Columns

The columns visible in tables and the order in which they are displayed can be configured using the **Configure Columns** option in the table shortcut menu.

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Change the left-to-right order in which columns are displayed using **Up** and **Down**. Alternatively, drag and drop table column headers to change column order.

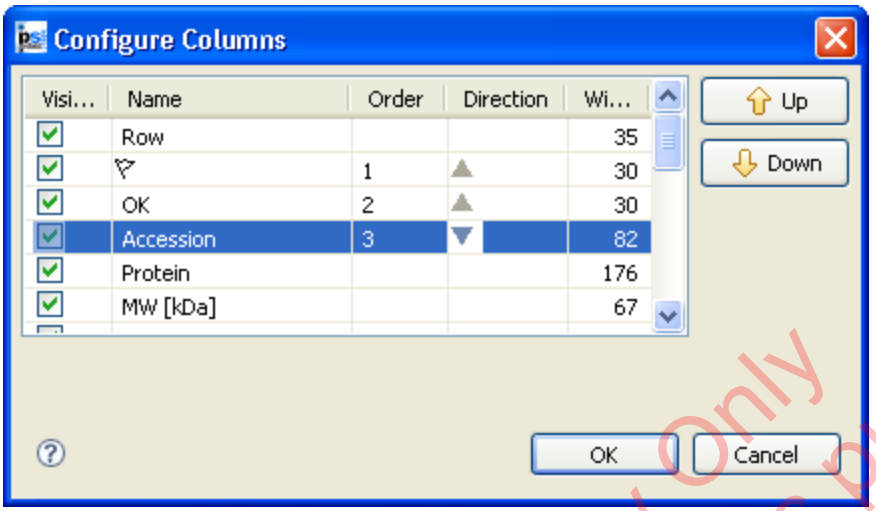

Figure 2-20 Configure Columns dialog

Table 2-5 Columns in the Configure Columns dialog

Column	Description
Visible	Check box to display parameter
Name	Parameter name
Order	Hierarchy of parameters used to sort columns. For instance, a protein list can be sorted on the basis of Score (**Order** =1) and then by molecular weight (**Order** = 2).
Direction	Switch column display order: ▲ low to high / A to Z ▼ high to low / Z to A
Width	Column width (pixels)

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Check/Uncheck OK State

The accepted status of one or more table entries can be defined by selecting or clearing the OK State option in the menu.

Flags

Colored flags can be added or removed from one or more table rows using the **Flags** option in the menu. The flags have no specific significance and can be used to label table rows for any reason.

Show

Additional Views can be launched using the **Show** option in the respective menu.

Select All

Selecting the **Select All** option in the menu highlights all entries in the table.

Excel Export

Automatically exports the current contents of the selected table to an Excel worksheet (see section 10.6).

Note When using this direct export functionality, all numbers are treated as text. This means that calculations or sorting in Excel may be problematic.

Copy to Clipboard

Enables the contents of the selected table to be pasted into spreadsheet applications (see section 10.7).

2.7 Zooming in on Graphic Images

Details in graphic views (for instance, Gel and Spectrum Views) can be enlarged using the view's zoom function. Clicking and dragging creates a framed zoom area that fills the view when the mouse button is released.

To reset the view:

Gel View: Click the **Zoom Original** button ( ) in the View toolbar.

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LC-MS Survey View: Right-click and select **Scaling > Reset** or double-click the View.

Quantitation Statistics View: Right-click and select **Show Original Size** or double-click the View.

Spectrum View: Select the **y-** and **x-axis autoscale control** check boxes in the lower-left corner of the View or double-click the View.

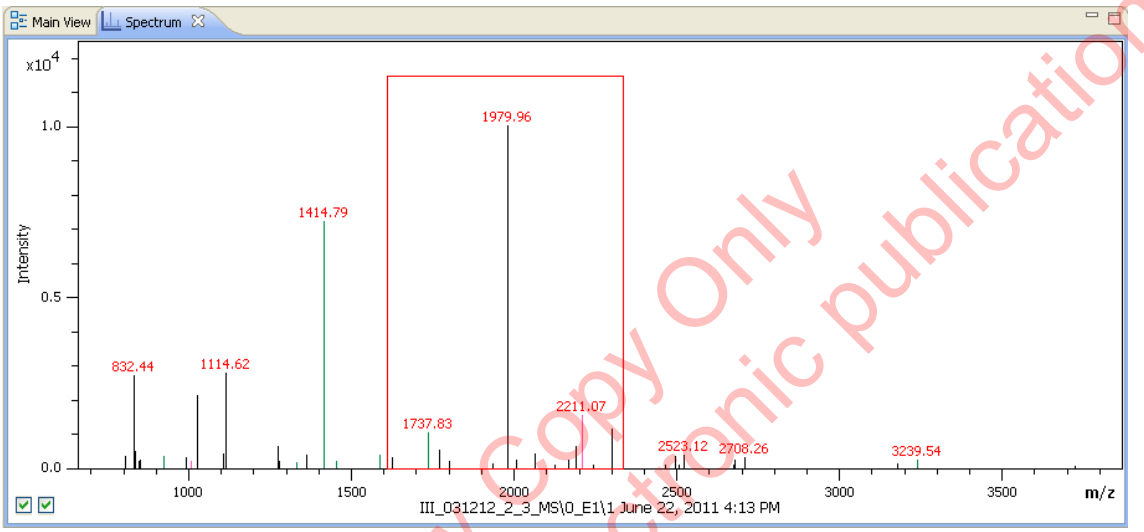

Figure 2-21 Zoom area selected by clicking and dragging

2.8 Context-Sensitive Help

ProteinScape provides an extensive context-sensitive Help function. Pressing **F1** displays a brief description of the context and links to the relevant topics in the Help View. Alternatively, the Help View can be opened by selecting **Window > Show View > Other > Help**.

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Table 2-6 Help View toolbar buttons

Button	Name	Action
	New window	Opens the Help in a new browser window
	Show in Contents	Highlights the current topic in the Help contents.
	Print	Opens a **Print** dialog for printing the current topic.
	Bookmark	Bookmarks the current topic.
	Highlight Search Terms	Highlights search terms throughout the Help.
	Back	Go one step back in a series of viewed topics.
	Forward	Go one step forward in a series of viewed topics.
	View Menu	Displays a list of additional commands.
	Related Topics	Opens the online help Related Topics page.
	Contents	Displays the online help contents as a tree diagram.
	Index	Opens the online help Index page.
	Search	Opens the online help Search page.
	Bookmarks	Opens the online help Bookmark page.
	Reduce/Increase Font Size	Reduces/Increases the font size on the online help pages.

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3 Navigating through the ProteinScape Database

The ProteinScape database contains projects and protocols, which are managed using the **Project Navigator** and **Protocol Navigator** respectively.

3.1 Expanding and Collapsing Nodes in Navigator Trees

Nodes in the **Project Navigator** and **Protocol Navigator** trees can be expanded or collapsed by double-clicking the node name or clicking the ⊞ or ⊟ button to the left of the node symbol.

Clicking **Collapse All** in the Project or Protocol Navigator toolbar collapses all nodes so that only top-level nodes are displayed.

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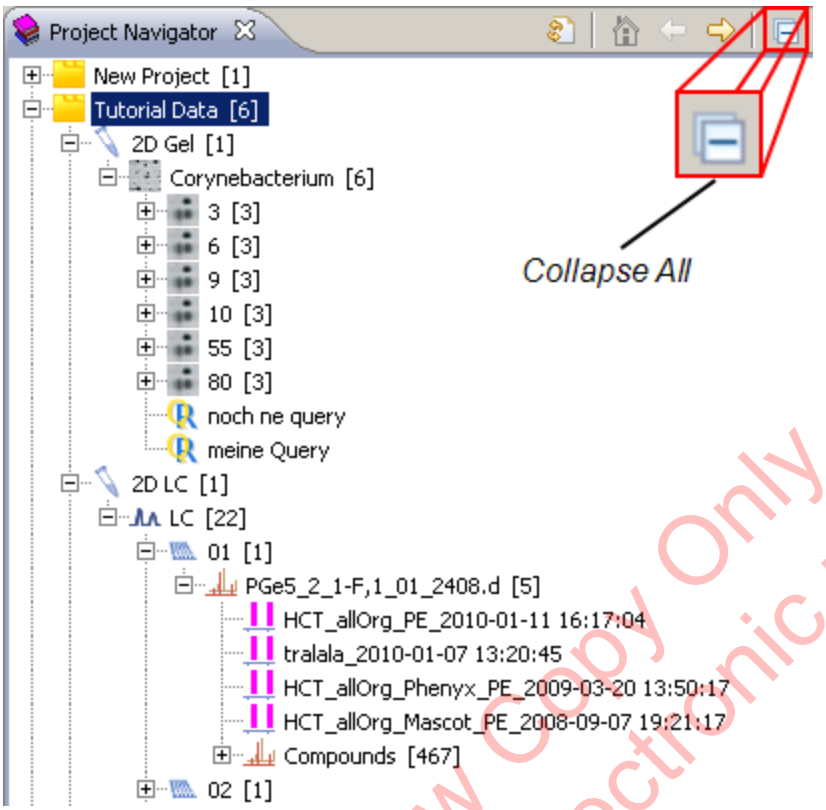

Figure 3-1 Example project containing 2D-gel and chromatographic separation

3.2 Organizing the Proteomics Workflow Using the Project Navigator

In the lab, a proteomics workflow consists of a series of different steps — for example, digestion, separation at protein and peptide level, and so on. In ProteinScape, such workflows are represented by **Project** sub-nodes in the **Project Navigator** tree.

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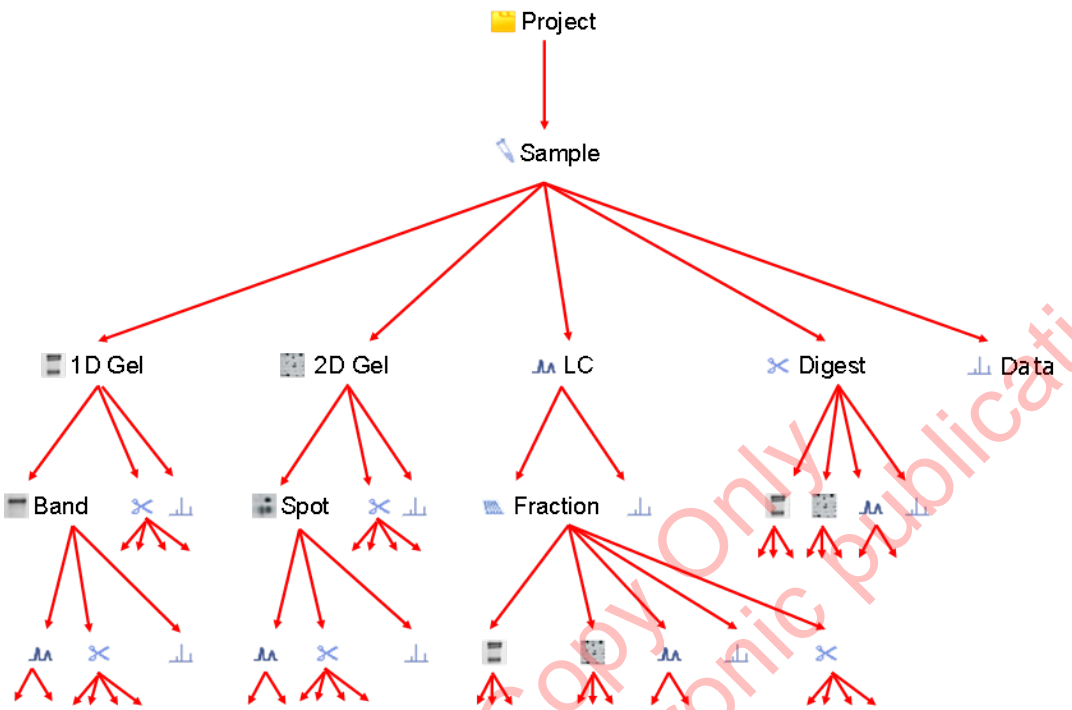

Figure 3-2 Proteomics workflow described in Project Navigator

The uppermost node of the tree is a **Project**, which contains one or more **Sample** nodes. A **Sample** node can contain separation and digestion information.

3.2.1 Project Navigator Node Hierarchy

Nodes in ProteinScape projects follow a defined parent/child hierarchy.

- Top-level nodes are Project nodes
- Second level nodes are Sample nodes
- Third- and lower-level nodes are Separation or Sample nodes

Project > **Sample** > Separation (**LC**, **1D Gel**, **2D Gel** or **Digest**).

The following figure shows an example **Project**. Two **Sample** nodes — labeled 2D Gel and 2D LC — are used to organize data from a two-dimensional (2D) gel and a 2D chromatographic separation.

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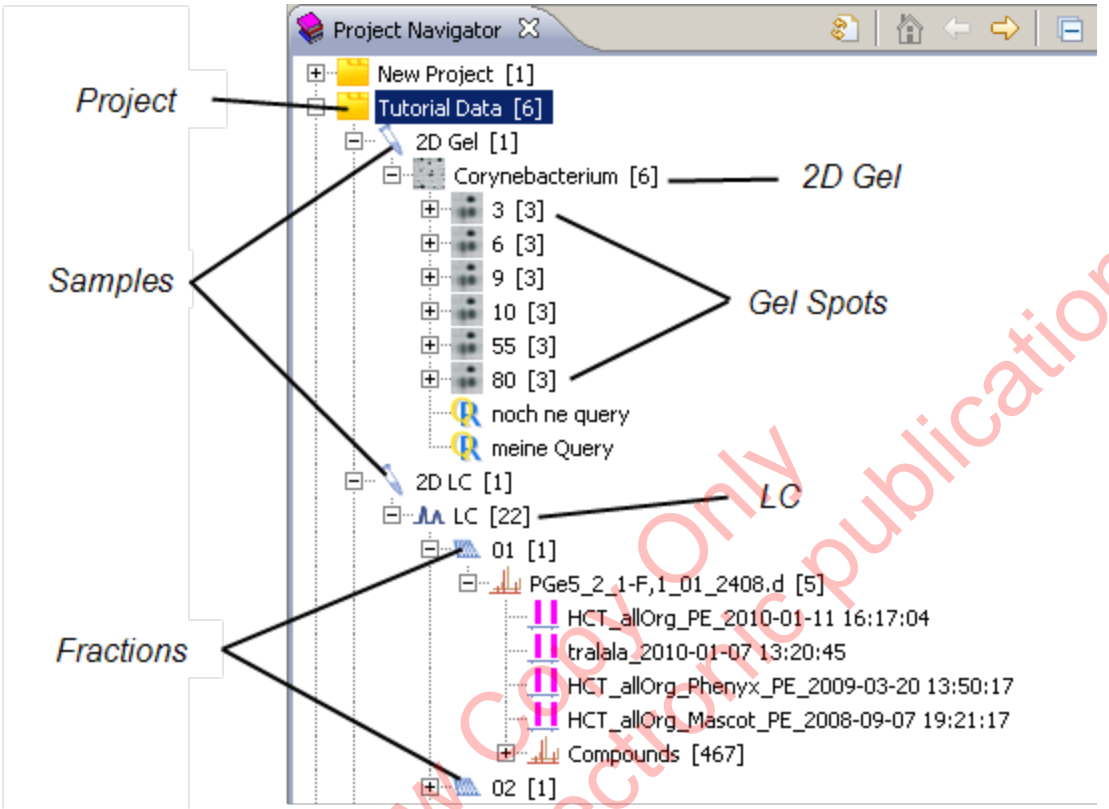

Figure 3-3 Project containing a 2D Gel and chromatographic separation

Project Navigator nodes have a defined hierarchy:

The following table displays valid child nodes for each node in the Project Navigator tree. MS data sets can be contained in any node.

3.2.2 Focusing on Individual Nodes

The **Project Navigator** toolbar buttons **Go Into**, **Back** and **Home** are used to focus on the sub-nodes of a particular node by hiding other nodes.

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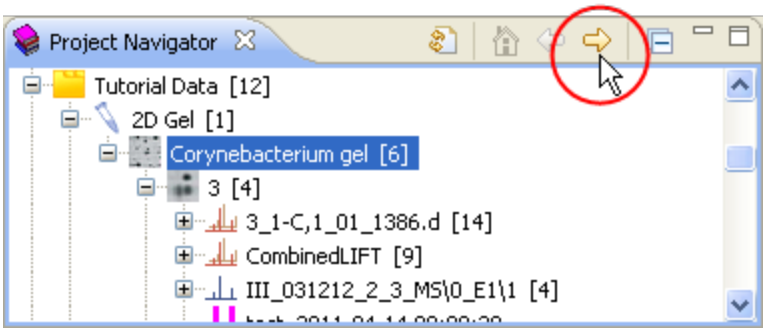

Figure 3-4 Click Go Into to focus on the contents of a selected node.

For example, if a **2D Gel** node is highlighted in the **Project Navigator** and the **Go Into** button is pressed,only the sub-nodes of the **2D Gel** node will remain visible. All other nodes will be hidden.

Clicking **Back** collapses the selected node and resets the View.

Clicking **Home** resets the View, making all nodes visible again.

Clicking **Collapse All** collapses all expanded nodes.

3.2.3 Project Navigator Tree Shortcut Menu

Each node in the **Project Navigator** has an associated shortcut menu, which is launched by right-clicking the node. The commands available in each shortcut menu depend on the node type and status.

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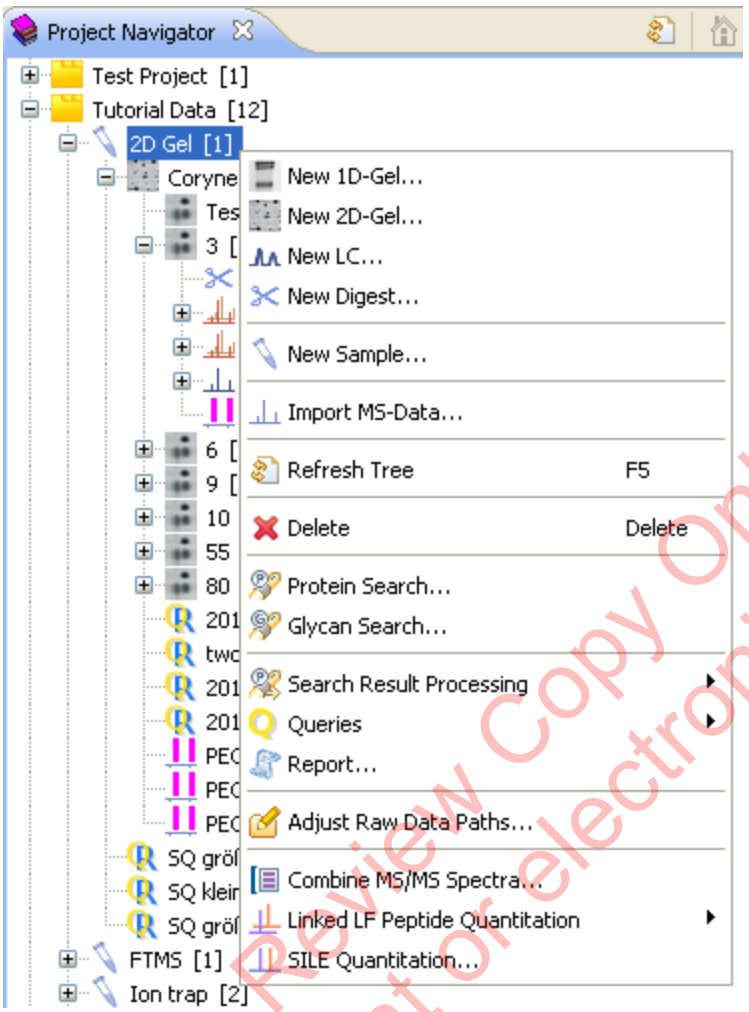

Figure 3-5 Sample node shortcut menu

3.3 Creating Project Nodes

A project node is created by selecting **File > New Project** or through the **Project Navigator** shortcut menu (right-click an existing project and select **New Project**).

Type a project **Name** (obligatory) and **Note** (optional) in the **New Project** dialog box and click the **Finish** button.

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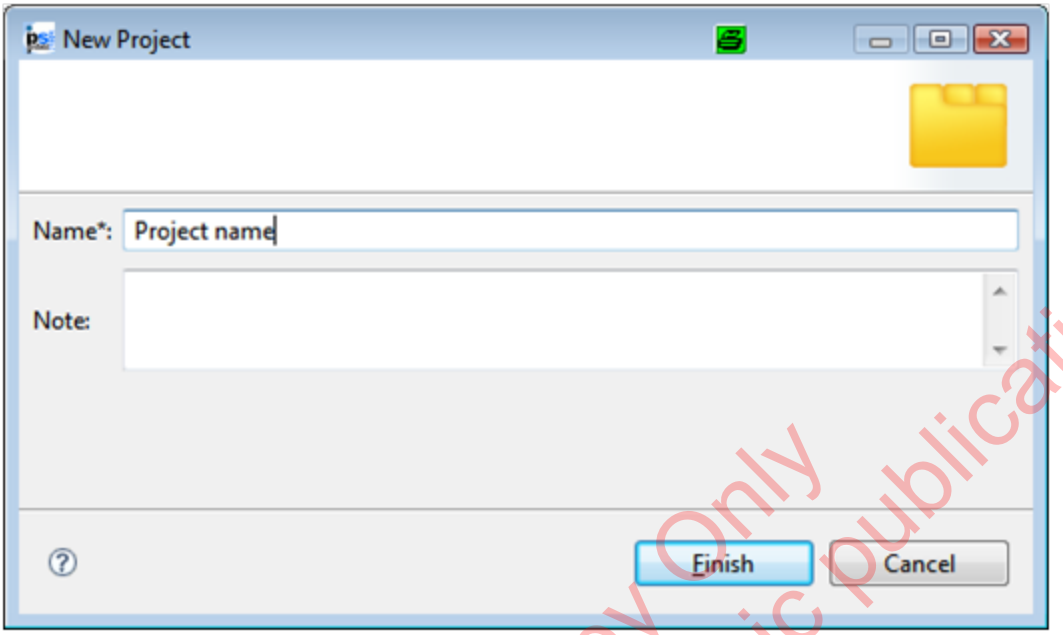

Figure 3-6 New Project dialog

3.3.1 Project Main View

Project-specific information is displayed in the **Project Main View**, which is divided into four areas:

- **Project Info**
- **Project Access**
- **Project Statistics** and
- **Project Parameters**.

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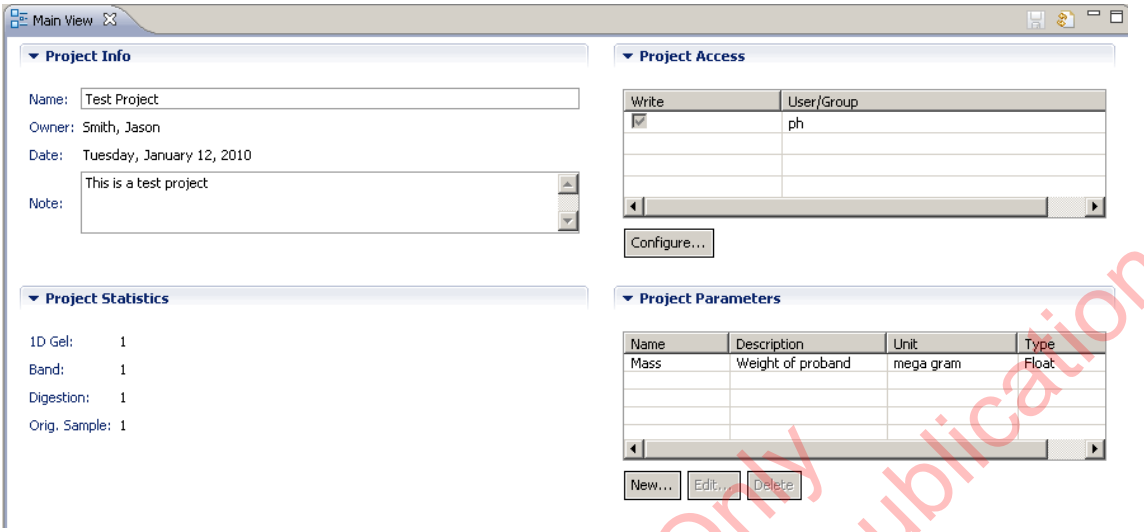

Figure 3-7 Project Main View

3.3.1.1 Project Info

The **Project Info** area of the **Project Main View** displays:

- the Project **Name**
- the Project **Owner** (the user who created the Project; added automatically)
- the **Date** (added automatically)
- the Project **Note** field.

The **Name** and **Note** parameters that were defined when the **Project** was created can be edited here.

3.3.1.2 Project Access

The **Project Access** area of the **Project Main View** displays the access rights of other users to the project.

Read and **Write** permissions can be assigned to users registered on the server by clicking **Configure** and selecting the relevant boxes in the **Configure Project Access** dialog box.

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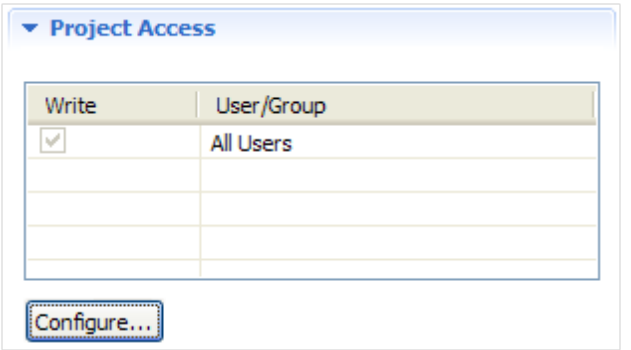

Figure 3-8 Project Access area of the Project Main View

Confirm changes by clicking the **OK** button.

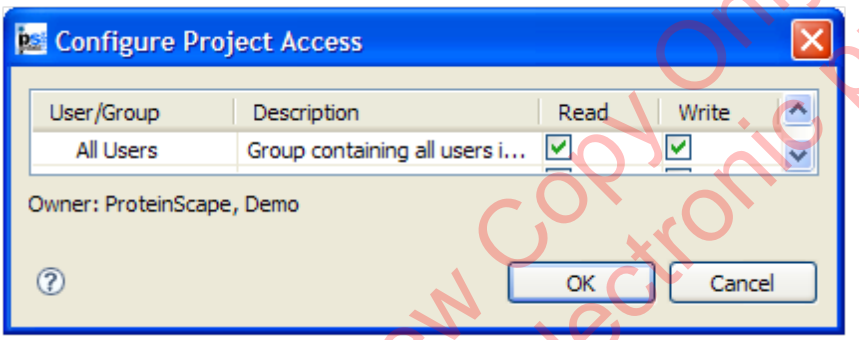

Figure 3-9 Configure Project Access dialog

Note A **User** who has access to a project might not have access to the protocols or search methods in the project.To provide a user with full access to a project, the owner must grant the **User** access to all protocols and search methods.

3.3.1.3 Project Statistics

Clicking **Show Statistics** in the **Project Statistics** area of the **Project Main View** displays a range of project information, for example, the number and types of separations and MS data, and the number of proteins and peptides identified.

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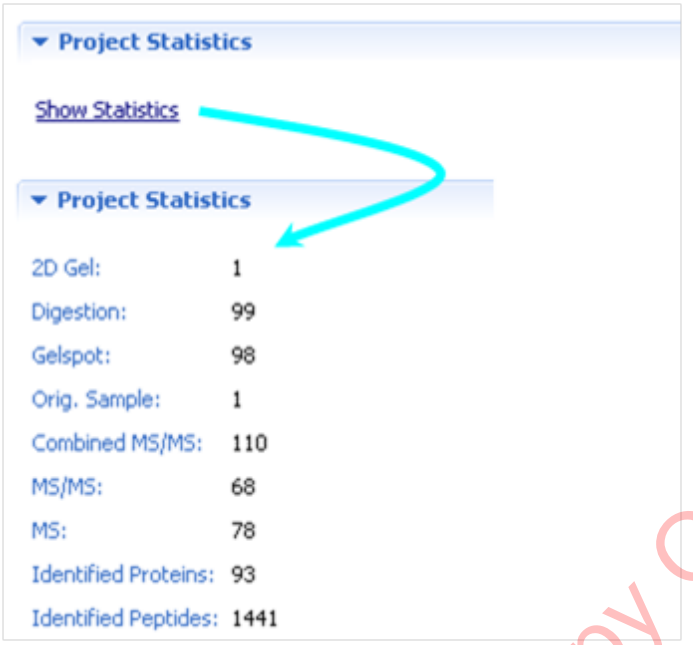

Figure 3-10 Project Statistics area of the Project Main View

3.3.1.4 Project Parameters

Project level parameters can be used to define specific information about a Sample in a Project.

For example, for a collection of human tissue samples, information on the date and time of collection and the health of the donors can be defined.

Project parameters can be used as input parameters for Queries (see section 9.1).

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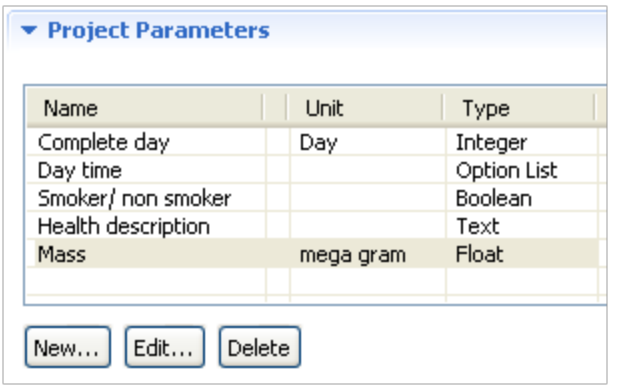

Figure 3-11 Project Parameters in the Project Main View

These parameters are used to generate a mask for the entry of the relevant information at the Sample level.

Creating Project Parameters

Click **New** to create Project level parameters for a group of samples. The **New Project Parameter** dialog box opens.

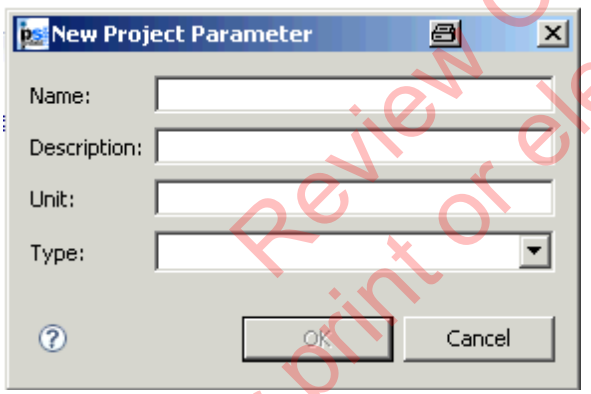

Figure 3-12 New Project Parameter dialog

Type or select the desired information in the following fields:

- **Name**
- **Description**
- **Unit** (optional)
- **Type** (see Table 3-1)

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Table 3-1 Project parameter types

Parameter type	Description
Integer	Requires insertion of an integer value in the Sample level entry mask.
Option List	Creates a drop-down list of options. Options can be added and removed using the **New** and **Remove** buttons accessed by clicking "…" in the Sample level entry mask.
Boolean	Allows **Yes** or **No** selection.
Text	Free text field.
Float	Requires insertion of a numerical value in the Sample level entry mask.

Editing Project Parameters

To change the **Description**, **Unit** or **Type** of an existing **Project Parameter**, select it in the **Project Parameter** list and click **Edit**.

The **Change Project Parameter** dialog box opens. Make the desired changes and click **OK**.

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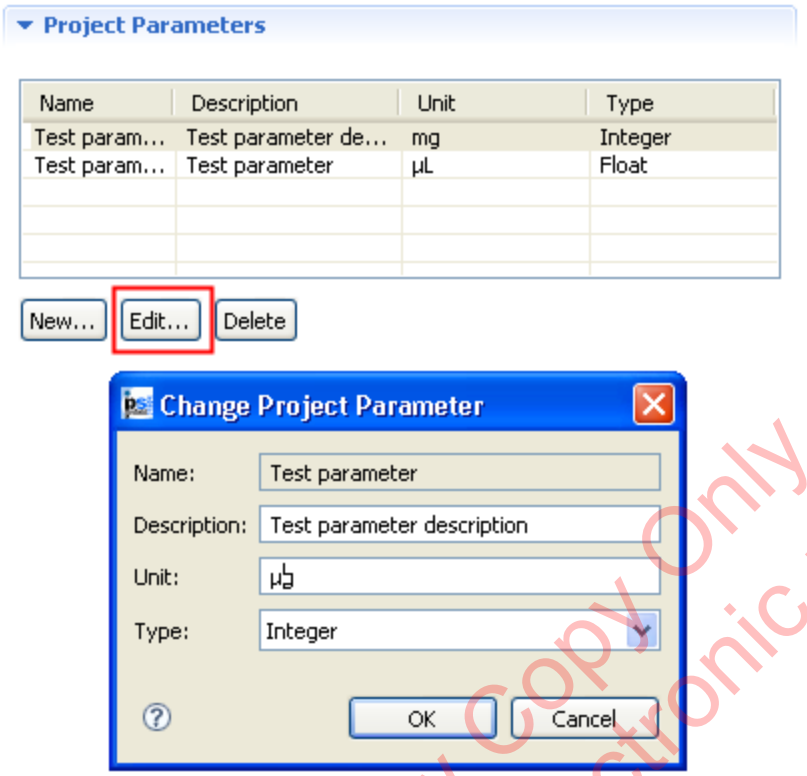

Figure 3-13 **Click Edit in the Project Parameters area of the Project Main View to open the Change Project Parameter dialog**

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3.4 Creating Sample Nodes

In ProteinScape, a Sample node is a representation of a laboratory sample that is subjected to an analytic workflow. To create a new Sample node, right-click the relevant Project symbol in the **Project Navigator** View and select **New Sample** from the shortcut menu.

Creating a new Sample node opens the **New Sample** dialog box. On the Sample Info page of this two-page dialog box, an obligatory **Name** and optional metadata (including the relevant values of the Project Parameters defined in the **Project Main View**) can be recorded.

Parameters on the **Project Parameters** page are configured in the Project Parameters area of the **Project Main View** (see section 3.3.1.4).

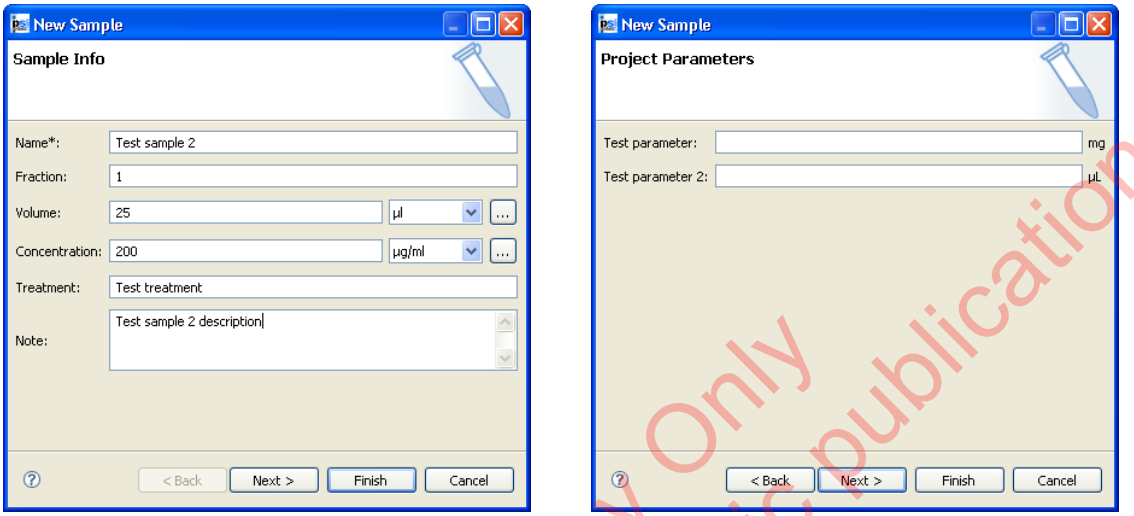

Figure 3-14 New Sample dialog box pages

Click the **Next** and **Back** buttons to switch between the dialog box pages. Click **Finish** to create the Sample node or **Cancel** to exit.

Information entered during creation of the node can be checked and edited in the node's **Main View**.

3.5 Creating Separation Nodes

Proteins contained in samples can be separated by applying separation techniques. These separations are represented in ProteinScape as:

- **LC**s consisting of **Fractions** (see section 3.5.1)
- **1D Gels** consisting of **Bands** (see section 3.5.2)
- **2D Gels** consisting of **Gel Spots** (see section 3.5.2)
- **Digests** (see section 3.5.3).

Each of these nodes is created using the shortcut menu within the **Project Navigator**.

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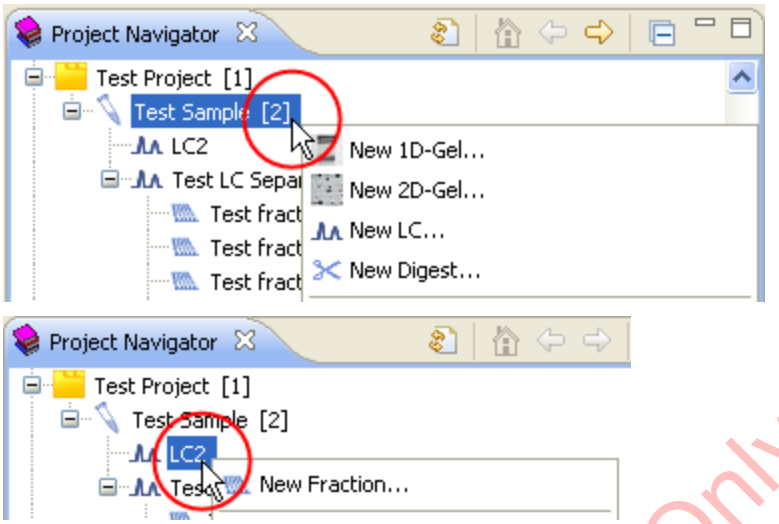

Figure 3-15 Creating separation nodes using the Project Navigator shortcut menu

Right-clicking the parent node and selecting the desired **New <node>** option will launch a multipage dialog box where the sub-node **Name** and metadata can be recorded (see Figure 3-16).

When creating second-level separation nodes (**Fraction**, **Gel Spot** and **Band**), the **No. of successive fractions/bands/spots** field can be used to generate multiple nodes labeled with **Name** followed by an incremented numerical suffix.

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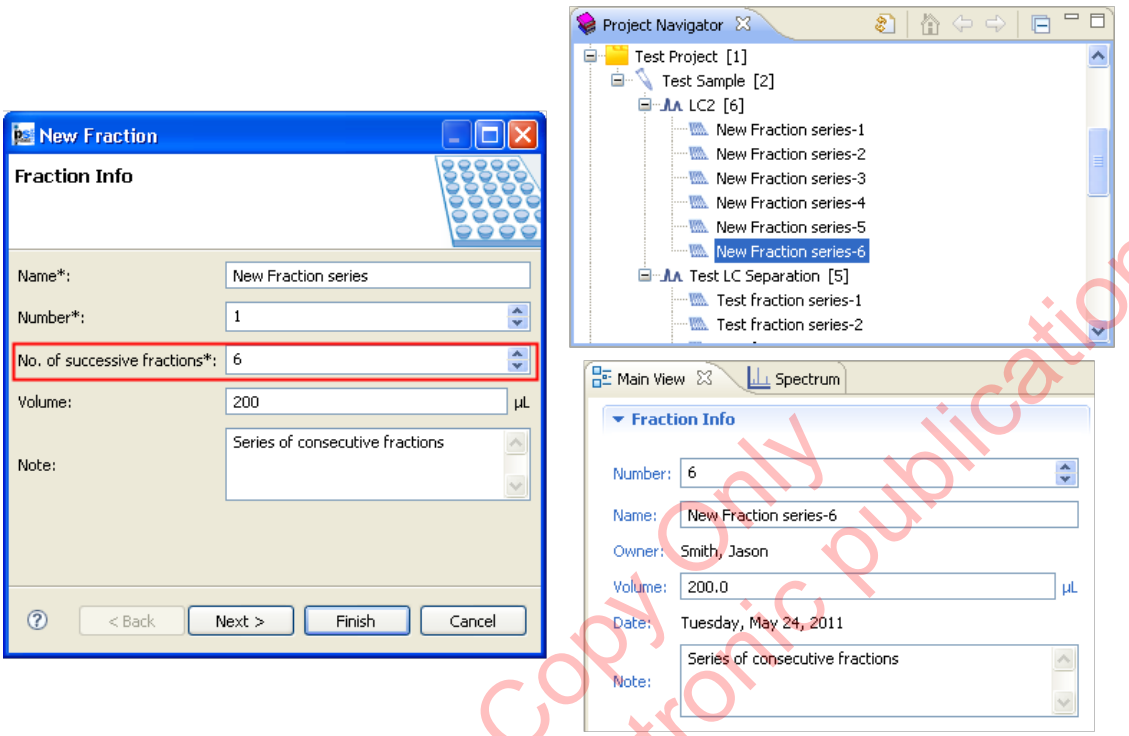

Figure 3-16 Creating a series of consecutive Fraction nodes in an LC node

Information entered during creation of the node can be checked and edited in the **<Node> Info** area of the node's **Main View**.

3.5.1 Liquid Chromatographic Separations — LC

The **LC** node can be used to represent any kind of liquid chromatographic separation. The associated metadata (visible in the LC Protocols area of the **LC** node's Main View) enables typical LC experimental parameters (for example, column type, elution gradient, buffer composition) to be recorded.

LC nodes can be sub-divided into one or more **Fraction** nodes, which in turn can be subdivided into other separation nodes to reflect downstream processing of samples (see Figure 3-17).

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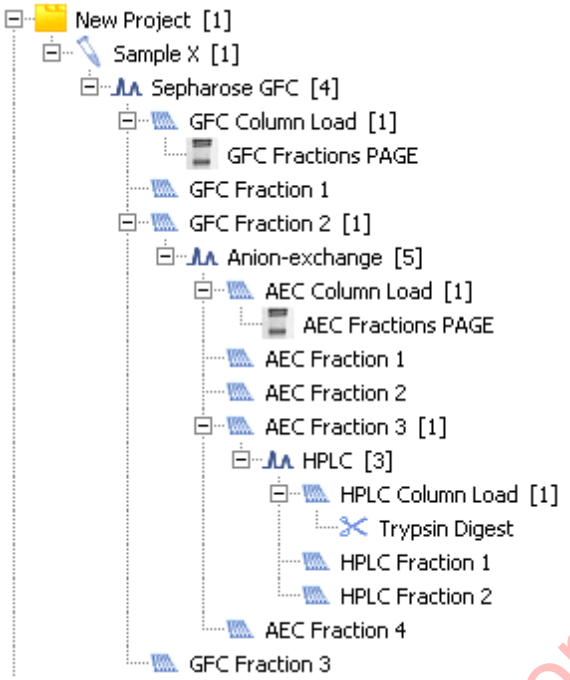

Figure 3-17 Example of a multi-column protein purification protocol recorded in ProteinScape

3.5.2 Electrophoretic Separations — 1D and 2D Gels

Gel nodes represent single- (1D) or two-dimensional (2D) gel electrophoretic separations, and are added to the **Project Navigator** tree to record the progress of protein purification.

3.5.2.1 Gel Image and Spot List Files

Gel image files can be added either during creation of the Gel node or by clicking **Import gel image and spot list** in the **Gel Info** area of the Gel node Main View. Supported image file formats are TIFF and JPEG.

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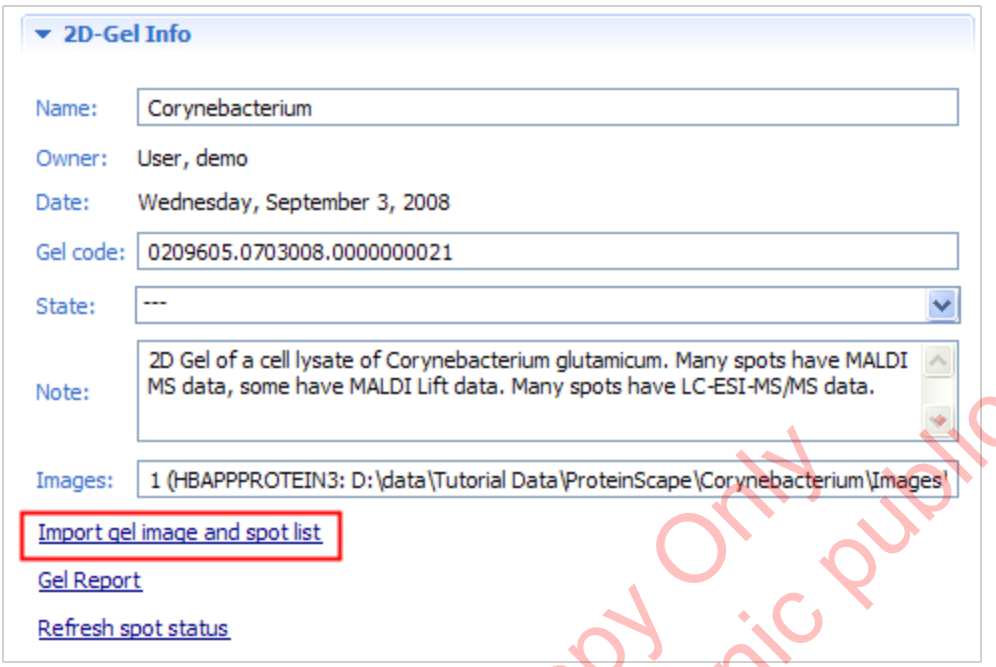

Figure 3-18 2D-Gel Info section of the Gel node Main View

Spot Lists

Spot list files can be added either during creation of 2D gel nodes or by clicking **Import gel image and spot list** in the **Gel Info** area of the node Main View

ProteinScape supports the following spot list formats:

- Bruker Proteineer SP format;
- ProteomWeaver (Bio- Rad), Delta2D (Decodon), Melanie/ImageMaster2D (GeneBio). Use the **Send to ProteinScape** button in the gel analysis software;
- Plain spreadsheet format (Excel or comma separated values):

The columns in the spot list spreadsheet must have the following headers:

- **x_coord**: x coordinate in pixels
- **y_coord**: y coordinate in pixels
- **no**: spot number
- **mw**: MW of the spot (if calibrated)

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- **pi**: pI of the spot (if calibrated)
- **area**: the area of the spot (if determined)
- **volume**: the volume of the spot = area x intensity
- **intensity**: the staining intensity of the spot

Please inquire about the compatibility of other spot list formats.

3.5.2.2 Creating Gel Band Nodes (1D Gels)

Band nodes are created by selecting **New Band** in the 1D Gel node shortcut menu within the **Project Navigator**.

Creating a new Band node opens the **New Band** dialog box.

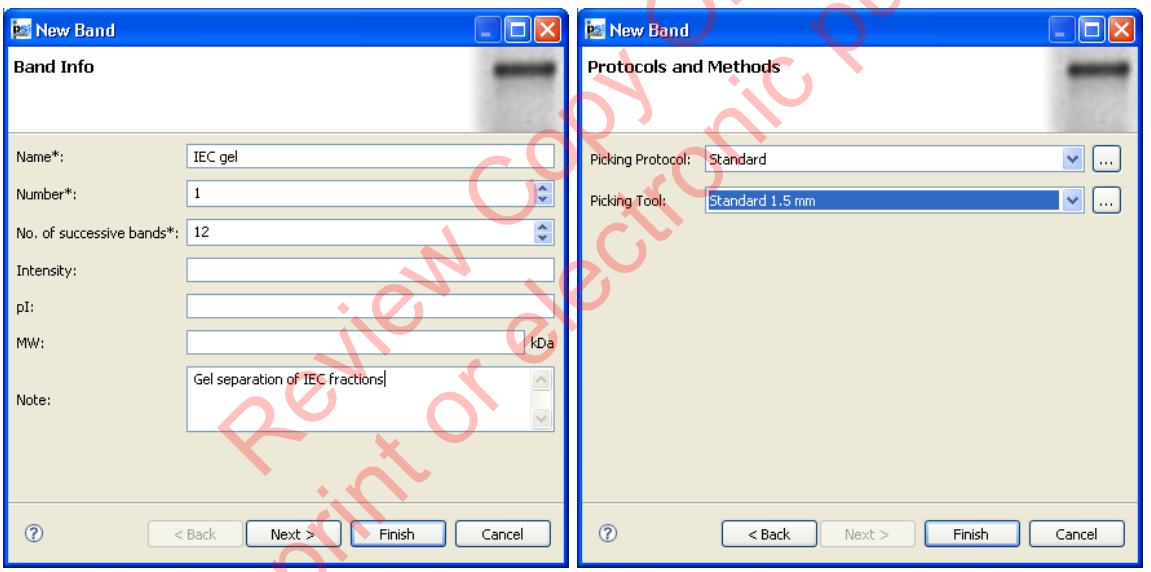

Figure 3-19 New Band dialog box pages

The Band Info page of the **New Band** dialog box contains fields for recording the name, separation number, number of bands, intensity, pI and molecular weight of bands and an optional description.

The **No. of successive bands** field can be used to generate multiple Band nodes labeled with **Name** followed by an incremented numerical suffix. The **Intensity**, **pI** and **MW** values

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of the individual bands can be defined and saved in the Band Info area of the Band node's Main View.

3.5.2.3 Creating Gel Spot Nodes (2D Gels)

Band nodes are created by selecting **New Gel Spot** in the 2D Gel node shortcut menu within the **Project Navigator**.

Creating a new Gel Spot node opens the **New Gel Spot** dialog box.

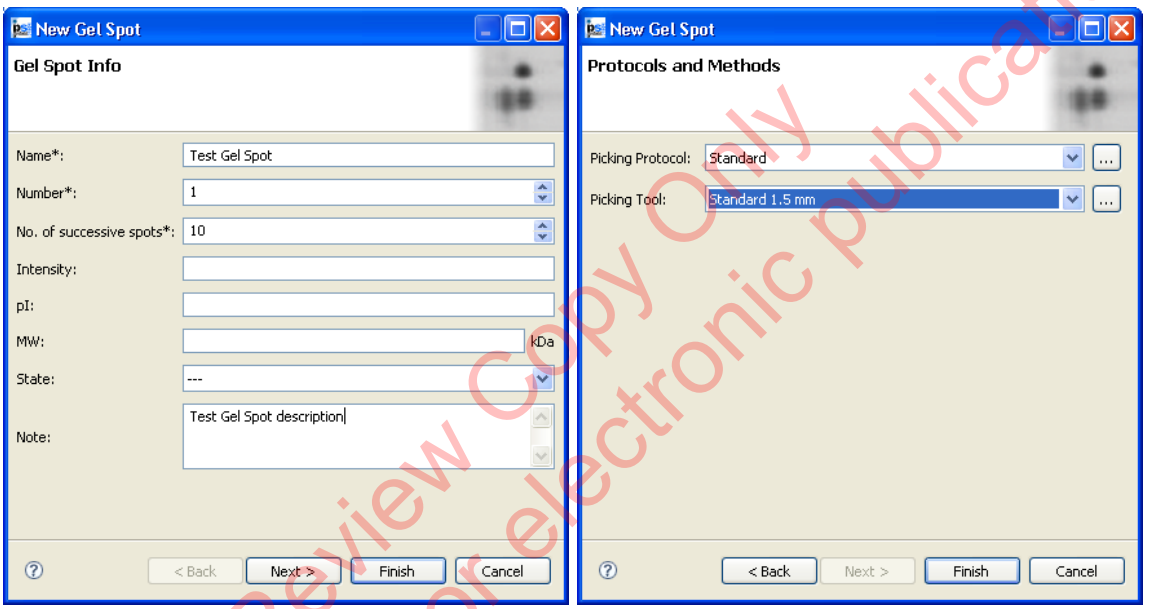

Figure 3-20 New Gel Spot dialog box pages

The Gel Spot Info page of the **New Gel Spot** dialog box contains fields for recording the name, separation number, number of successive spots, intensity, pI, molecular weight and status of spots and an optional description.

The **No. of successive spots** field can be used to generate multiple Gel Spot nodes labeled with **Name** followed by an incremented numerical suffix. The **Intensity**, **pI**, **MW** and **State** values of the individual spots can be defined and saved in the Gel Spot Info area of the Gel Spot node's Main View.

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3.5.3 Digests

The Digest node is used to indicate a proteolytic digest in a proteomic workflow. Details of the enzyme used and its cleavage activity can be recorded during creation of the node and edited in the Digest node's Main View.

3.5.4 Alternative Protein and Peptide Separation Methods

Other methods used for protein and peptide separations can be represented using ProteinScape nodes.

Table 3-2 Suggested node representations of separation methods

Method	Suggested node
IEF	1D Gel or 2D Gel
Western Blot	1D Gel or 2D Gel
Ammonium Sulfate Precipitation	LC
Subcellular Fractionation	LC

3.6 Deleting Project Navigator Nodes

To delete a node in the **Project Navigator** tree, right-click the node, select **Delete** from the shortcut menu, and confirm the action.

Note Deleting a node will mean that all sub-nodes of the selected node will also be deleted.

Because deleting large amounts of data can lead to a loss of processing speed, in most cases, "deleted" Project nodes are hidden and queued for deletion overnight. This background process can be configured in the Administrator Preferences window (see *ProteinScape Administrator Manual*).

3.7 Using the Protocol Navigator

The Protocol Navigator is used to store parameter settings and facilitates addition of metadata to new nodes (see section 3.7.1).

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For instance, column configurations defined in the Protocol Navigator **Column Configuration** dialog (see Figure 3-21) are available for selection when new LC nodes are created and are also added to the drop-down list in the **Column** field in the LC Protocols area of the Main View (see Figure 3-22). This ensures consistent annotation of multiple LC separations performed using the same column.

Nodes in the Protocol Navigator tree correspond to node types in the Project Navigator (for example, Sample, Fraction and LC).

Each node contains one or more attributes (for example, the LC node contains the attributes Column, LC Protocol, LC Technique and Solvents).

Each attribute contains one or more parameter-based configurations (for example, Column configurations are defined using the parameters Pore Size, Diameter and Length).

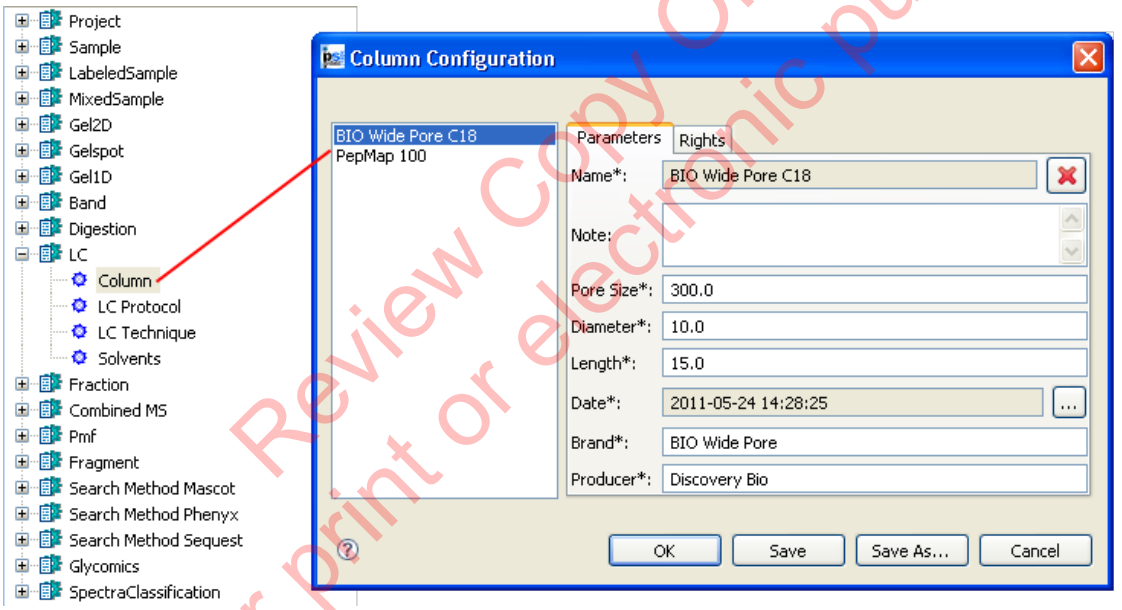

Figure 3-21 Parameters that define configurations of the Column attribute

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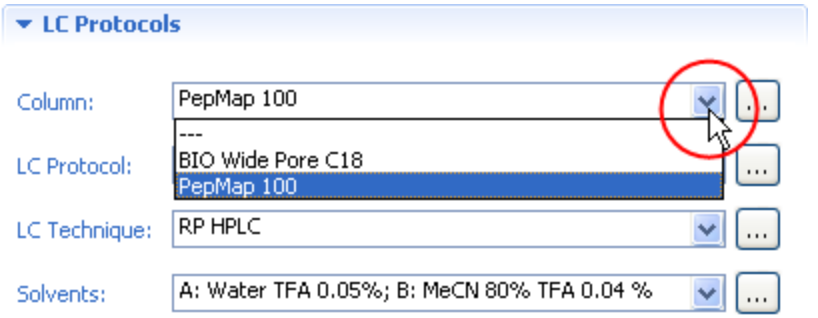

Figure 3-22 Column configurations in the LC Protocols area in the LC Main View

The Protocol Navigator is also used to control user access to protocol information through the **Rights** tab (see section 3.7.6).

The Protocol Navigator is accessed through **Window > Show View > Protocol Navigator**. Nodes in the Protocol Navigator tree can be expanded or collapsed by double-clicking the node name or clicking the " + " or " – " sign in front of the protocol symbol.

3.7.1 Protocol Navigator Node Attributes

The following table lists the attributes that can be defined for each node in the Protocol Navigator.

Node	Attributes	Node	Attributes
Project	Project part-ner	**LC/Combined MS**	Column
Sample/Labeled Sample	Buffer		LC Protocol
	Cell Line		LC Technique
	Concentration Protocol		Solvents
	Label		MALDI Preparation (Combined MS only)
	Organ	**Fraction**	Solvents
	Organism	**Pmf**	MALDI Preparation

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Node	Attributes	Node	Attributes
	Pathology	**Fragment**	MALDI Preparation
	Purification	**Search Method Mascot/Phenyx/Sequest**[1]	Cleavage Enzyme[2,3,4]
	Source		Modification[2,3,4]
	Subcellular Fraction		Sequence Database[2,3,4]
Mixed Sample	WARP-LC Method		Mass Spectrometer Type[2,3]
Gel2D	IEF Protocol		Default Parent Charge[2,3]
	PAA Protocol		Taxonomy[2,3]
	Stain Protocol		Ion Series[2]
Gelspot	Picking Protocol		Peptide Mass Tolerance unit[2]
	Picking Tool		Scoring Model[2]
Gel1D	Separation Protocol		Turbo Error Tolerance unit[2]
	Stain Protocol		
Band	Picking Protocol	**Glycomics**	GlycoAssessment
	Picking Tool		GlycoComposition
Digestion	Cleavage Enzyme		GlycoFragmentation Type
	Digestion Protocol		GlycoSearch Method
	Enzyme Lot		GlycoSearchParams

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Node	Attributes	Node	Attributes
			GlycoTaxonomy
		SpectraClassification	MassDistances
			MassPattern
			MassSignals
			SpectraClassification

[1] *See section 3.7.5 for further details;* [2] *Phenyx;* [3] *Mascot;* [4] *Sequest*

3.7.2 Editing Protocol Configurations

►► To edit a protocol configuration

1. Double-click the desired protocol attribute (for example, **Organism**).
2. Select the configuration to be changed (for example, **Bos taurus**) in the configuration list and make the desired changes to the respective parameters.
3. Click **OK** to save the changes.

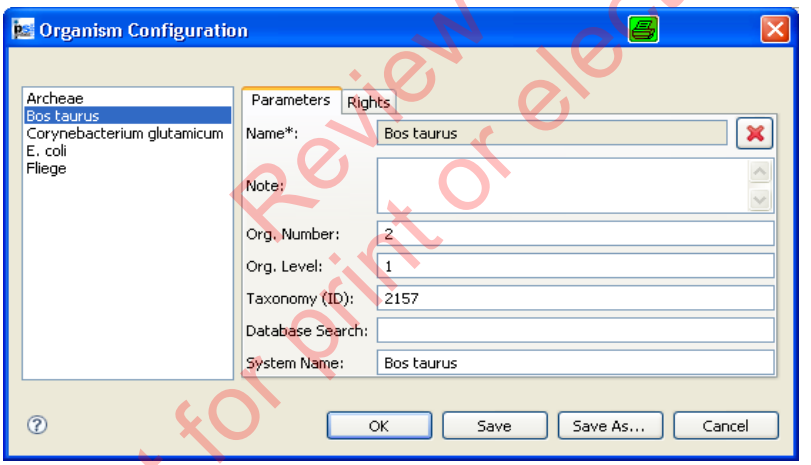

Figure 3-23 Organism > Bos taurus parameters

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3.7.3 Creating Protocol Configurations

►► To create a protocol configuration

1. Double-click the desired attribute (for example, **Organism**).
2. Select an existing configuration (for example, **Chicken**) in the configuration list and click **Save As**.
3. Type the new configuration name in the **Name** field.
4. Click **OK** .
5. Select the new configuration in the configuration list and make the desired changes to the relevant parameters.
6. Click **OK** to save the changes and exit the dialog.

Note When a new protocol configuration is created, only the user who created the configuration has access rights. The user rights must be changed to make the new protocol configuration available to other users (see section 3.7.6.1).

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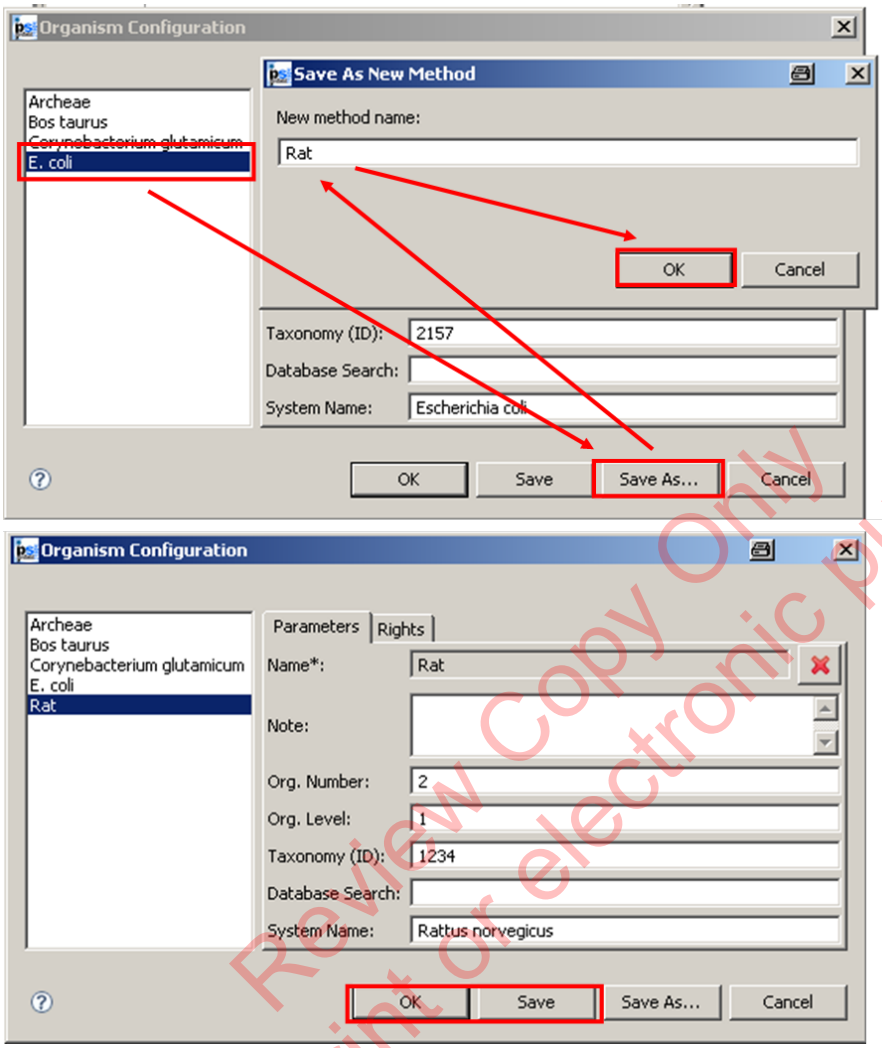

Figure 3-24 Steps in creating a protocol configuration

To add and use search-engine specific parameters such as databases, the parameter must be introduced into the search engine database. For more information, see the respective search engine documentation.

ProteinScape and the search engines must be resynchronized by selecting the respective search engines in the **Search Engine Settings** dialog box and clicking **Resynchronize now**.

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After synchronization, all databases available on the search engine will also be available in the **Search Method** protocols. Configurations in the **Search Method** protocols (for example, **Sequence Database**) should not be edited manually.

3.7.4 Deleting Protocol Configurations

►► To delete a protocol configuration

1. Double-click the desired attribute (for example,**Organism**).
2. Select an existing configuration (for example, **Chicken**) in the configuration list and click .
3. Click **Yes** when prompted to confirm deletion.

3.7.5 Creating Search Engine-Specific Parameters

Search engine-specific parameters are automatically synchronized with the respective search engine when **Resynchronize Now** is clicked in the **Search Engine Settings** dialog box. The mapping between the search engine's modifications and ProteinScape's (unimod-based) modifications is usually generated automatically during this process.

For validation purposes, the search engine's modifications (for example, **Modification Mascot**) and the reference's unimod modifications can be edited using the **Protocol Navigator**.

3.7.6 Protocol User Rights

User access to Protocol parameters can be controlled through their **Rights** tab. Clicking the configuration name in the list displays the valid **Write** permissions.

Click **Configure** to change **Write** permissions and **OK** to save changes.

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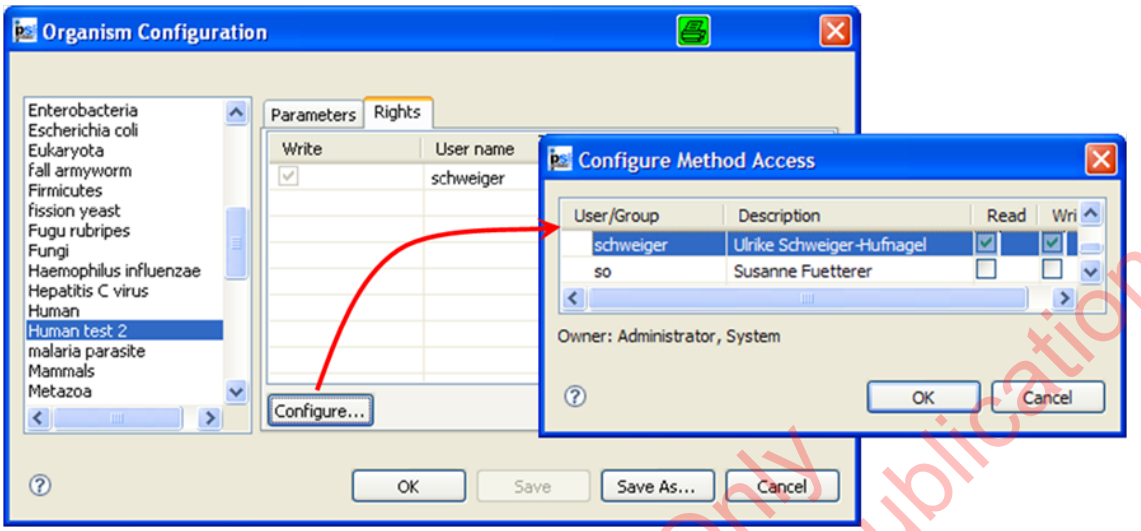

Figure 3-25 Organism Configuration dialog

When a new parameter — for example, a new Mascot Sequence Database entry — is created, the “owner” has exclusive access rights and the new parameter is invisible to other users until its **Rights** properties are changed, for example, to “All users”.

3.7.6.1 Assigning Protocol Configuration User Rights

User rights for attribute configurations are assigned on the respective configuration's **Rights** tab.

Note When a new protocol configuration is created, only the user who created the configuration has access rights. Use the following procedure to make the new protocol configuration available to other users.

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►► To assign protocol configuration user rights

1. Click the relevant node attribute to open the protocol configuration dialog box.
2. Click the **Rights** tab to open the **Rights** dialog box.
3. Click **Configure** to display a list of all users and groups currently registered on the ProteinScape server.
4. Select the desired user(s) and/or group(s) and assign read/write permissions by selecting the relevant check box(es).
5. Click **OK** to save changes and exit the dialog.

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4 Handling MS Data

Three types of MS data set can be imported into ProteinScape:

- Single MS spectra (for example, MALDI TOF PMF)
- Single MS/MS spectra (for example, MALDI TOF/TOF MS/MS)
- Combined data, which can be:
  - multiple MALDI TOF/TOF MS/MS spectra
  - MS/MS spectra without LC context (for example, a plain `.mgf` file)
  - an LC run containing a number of MS/MS spectra with or without the associated MS spectra (for example, a Bruker DataAnalysis XML file)

  Combined MS/MS data sets contain a sublevel containing individual compounds. An individual compound (Cmpd) contains:

  - A precursor mass (*m/z*)
  - A retention time (min, optional)
  - An MS spectrum (optional)
  - One or more MS/MS spectra (usually one CID or LID spectrum [which can be the sum of several raw spectra] and/or one ETD or ECD spectrum).

4.1 Importing MS Data

MS data sets can be imported into any of the following nodes in the **Project Navigator** tree:

- Project
- Sample
- Digest
- 1D Gel and Band
- 2D Gel and Spot
- LC and Fraction

Data can either be imported manually (see section 4.1.1) or automatically (see section 4.1.2).

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4.1.1 Manually Importing MS Data

►► To manually import MS data into a node in the Project Navigator tree

1. Right-click the respective node and select **Import MS-Data** from the shortcut menu.

   The **Import MS-Data** dialog box opens. The data imported into the selected node can be filtered by selecting the desired **Input Options**. The **Input Options** and **Default Configuration** areas can be collapsed and expanded using the « button in the upper-right corner of the respective area.

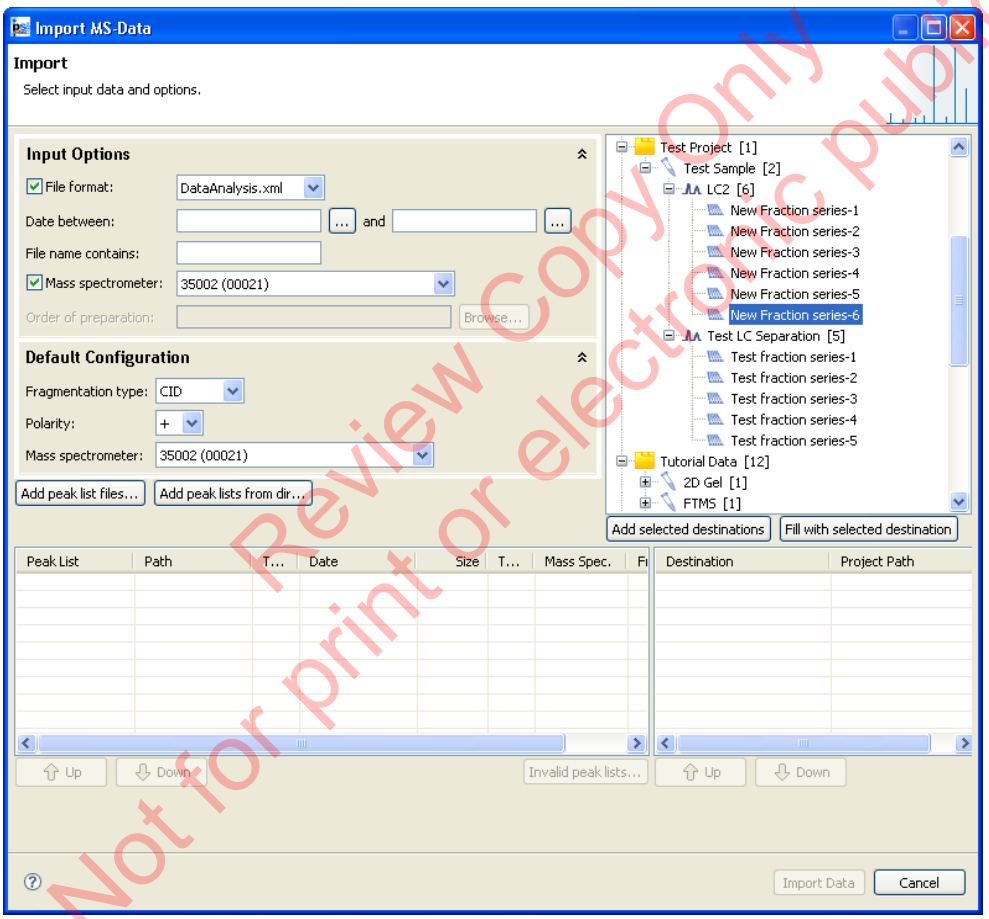

Figure 4-1 Import MS-Data dialog box

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2. Select the desired MS data input options in the **Input Options** area of the dialog.

   These options can be used to filter for data sets that meet the desired criteria when adding peak list files to the peak list table.

   a. Select the **File format** check box and select the desired format from the drop-down list. Supported **Formats** are:

      - Data Analysis.xml
      - MALDI PMF+ LIFT - flexAnalysis, single MS (PMF) spectra (peaklist.xml) or flexAnalysis, single Lift (MS/MS) spectra (peaklist.xml. These will be combined into a single combined MS/MS data set.)
      - DTA - single dta files (will be combined into a single combined MS/MS data set.)
      - MGF – for example, merged dta files as generated by Mascot merge.pl
      - PKL files

   Note Bruker LC-MALDI runs cannot be imported into ProteinScape manually. They must be sent to ProteinScape by WARP-LC.

   b. Data recorded over a specific period of time can be imported by defining the start and end points in the **Date between** fields. Click the **…** buttons to open a calendar and time field.

   c. The **File name contains** field enables import of files whose name contains a defined string.

   d. Assign a mass spectrometer to a data set (if required).

   Most Bruker peak list files contain information about the mass spectrometer used to acquire the data. This information is read by ProteinScape, and the **Mass Spectrometer** parameter is automatically assigned to the respective data sets.

   For peak list files that do not contain this information (for example, `*.mgf` files) the mass spectrometer can be selected manually. Select the Mass Spectrometer check box and select the desired mass spectrometer from the drop-down list.

   e. Define an **Order of preparation** file.

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The **Order of preparation** parameter is exclusive to MALDI experiments and sets an order of preparation for the target positions. This order is specified in an XML file. This greatly facilitates the mapping of target positions for example, to gel spot numbers. The general concept and format of the XML file is exactly the same as that of the AutoXecute Run Editor. Detailed information can be found in the *AutoXecute Run Editor User Manual*. An example of a valid XML file is:

```
<sequence>
<pos spot ="A1" chip="0" />
<pos spot ="C1" chip="0" />
<pos spot ="E1" chip="0" />
<pos spot ="G1" chip="0" />
<pos spot ="I1" chip="0" />
<pos spot ="K1" chip="0" />
<pos spot ="M1" chip="0" />
<pos spot ="O1" chip="0" />
</sequence>
```

3. Select the desired default configuration values in the **Default Configuration** area of the dialog.

   Default configuration values for **Fragmentation Type**, **Polarity** and **Mass Spectrometer** can be selected from the respective drop-down list. These values will be used for every data set that does not contain this information in the peak list, for instance, MGF files.

Figure 4-2 Defining the default configuration for manual MS data import

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4. Add peak list files to the peak list table.

   a. Click the **Add peak list Files** or **Add peak lists from dir** buttons to add peak list files individually or as batches from directories respectively. Clicking either button opens a Windows Explorer window where the desired file/directory can be selected.
   b. Click **OK** to import files.
   c. Alternatively, peak lists can be dragged from Windows Explorer into the table. However, the drag-and-drop option is not compatible with the external order of preparation function using an XML file (see above).

   Data in the peak list table can be sorted using any column by clicking the column header.

   To delete data sets from the peak list table, select and right-click the unwanted data set(s) and select **Remove peak lists from list** from the shortcut menu.

   Use the **Up** and **Down** buttons to move individual data sets within the list (not possible after sorting).

   Click **Invalid peak lists** to highlight all invalid peak lists in the selected directory. These will not be imported into ProteinScape.

5. Select one or more destination nodes for the imported data.

   a. Select the desired destination node(s) in the Project Navigator tree in the **Import MS-Data** dialog.

   Select multiple nodes by holding **SHIFT** (consecutive entries) or **CTRL** (individual nodes) when making selections.

   b. Click **Add selected destinations** to add the selected nodes to the Destination table.

   Entries in the **Destination** table can be sorted using any column by clicking the column header.

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To delete nodes from the **Destination** table, select and right-click the unwanted node(s) and select **Remove analytes from list** from the shortcut menu.

Use the **Up** and **Down** buttons to move individual nodes within the list (not possible after sorting).

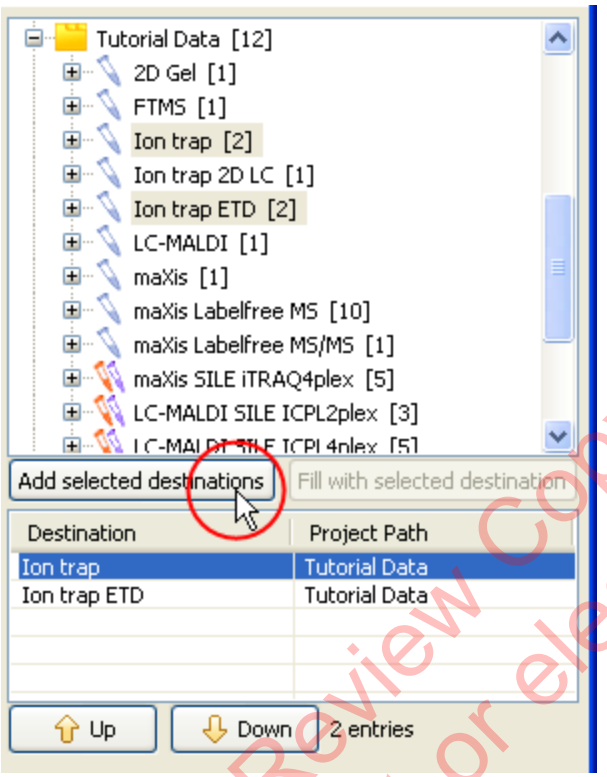

Figure 4-3 **Adding destination nodes to the Destination table in the Import MS-Data dialog**

Optional: To assign multiple peak list files to the same destination node, add the peak list files to the **Peak List** table, select the desired destination node and click **Fill with selected destination**. The selected destination node will be added to all positions in the **Destination** table. **Fill with selected destination** is not available if more than one node is selected in the Project Navigator tree in the **Import MS-Data** dialog.

6. Click **Import Data** to import the peak list files in the **Peak List** table into the corresponding nodes in the **Destination** table.

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4.1.2 Automatically Importing ESI MS Data

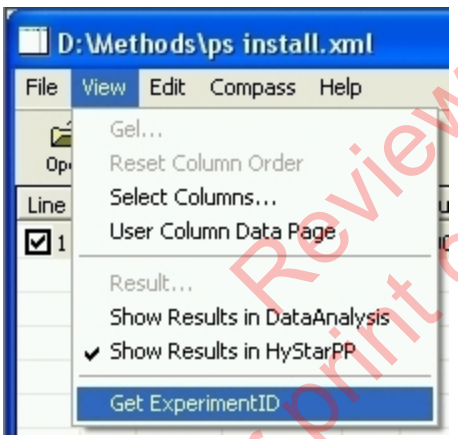
The Bruker Daltonics Push Daemon is used to transfer MGF peak lists and XML peak lists created by DataAnalysis to ProteinScape. The Push Daemon must be configured before data can be imported.

4.1.2.1 Assigning Spectra to a ProteinScape Node Using HyStar

All types of Bruker ESI mass spectrometers can be integrated into an automatic workflow. Experiments must be set up in the HyStar sample table (see 4.1.2.1). In addition to the standard columns required to set up an LC-MS/MS experiment, the **ExperimentID** column must contain the relevant entry. Before spectra are automatically transferred to ProteinScape using the Push Daemon, the target node where spectra will be saved must be defined in HyStar.

►► To assign spectra to a target node using HyStar

1. Open HyStar and select **View > Get ExperimentID**.



2. Log in to ProteinScape and select the destination node to which spectra will be transferred.

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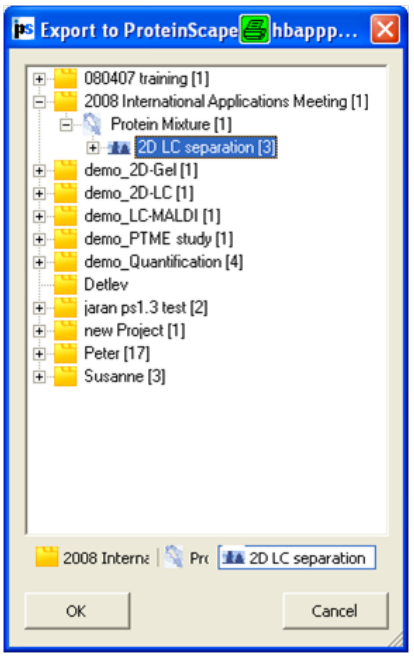

Figure 4-4 Selecting a target node in the HyStar Export to ProteinScape dialog

The relevant experiment ID appears in the HyStar sample table.

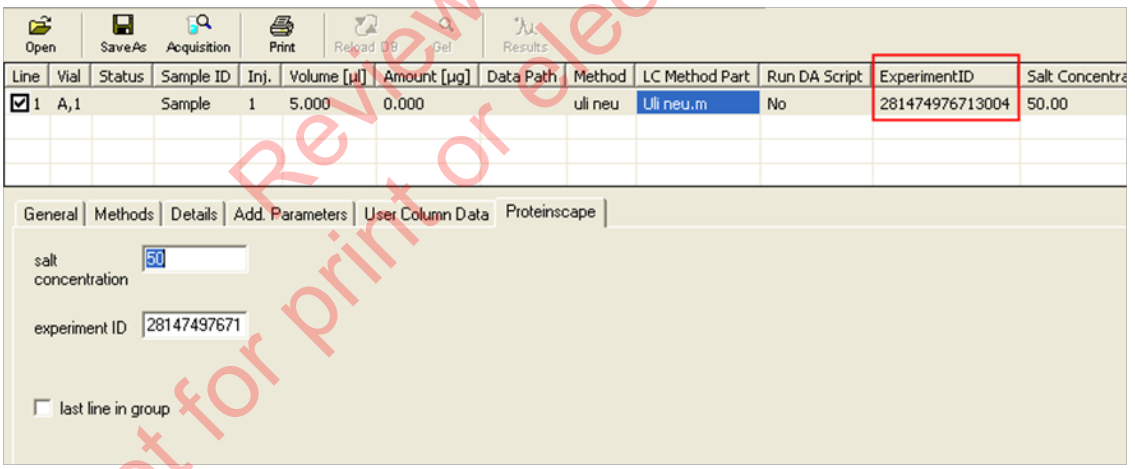

Figure 4-5 Experiment ID appears on the ProteinScape page in HyStar

3. Open the **Methods** page, select **Use Method** and select a suitable DataAnalysis method that creates MGF and/or XML spectra.

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4. Select **Run Script** on the **Methods** page in HyStar.

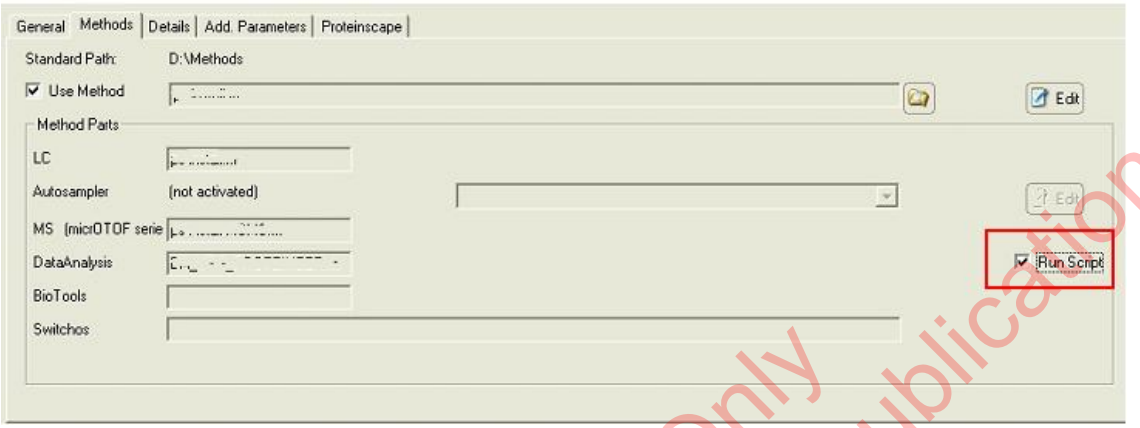

Figure 4-6 Run Script selected on the Methods page in HyStar

Optional: Select **View > Select Columns** to open the **Column Selection** dialog. Select **Experiment ID** and **Run DA Script** to display the relevant information in the HyStar Sample Table.

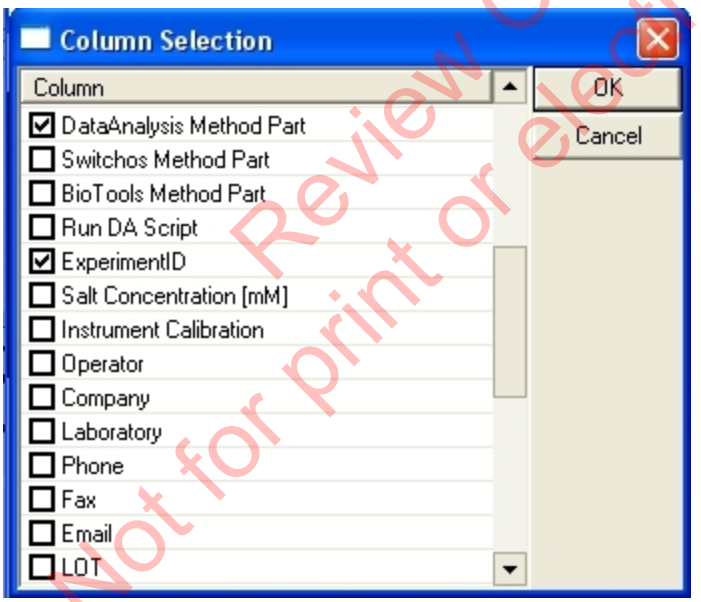

Figure 4-7 Column Selection dialog

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4.1.2.2 Configuring the Push Daemon

►► To configure the Push Daemon

1. Make sure that the Push Daemon has been installed on the data processing computer (see section 1).
2. Select **Start > All Programs > Bruker Daltonics > Utilities > Bruker Push Daemon** to start the Push Daemon.

   The Push Daemon symbol appears in the task bar.
3. Click the **Directories to Watch** tab and the **Add directory** button.

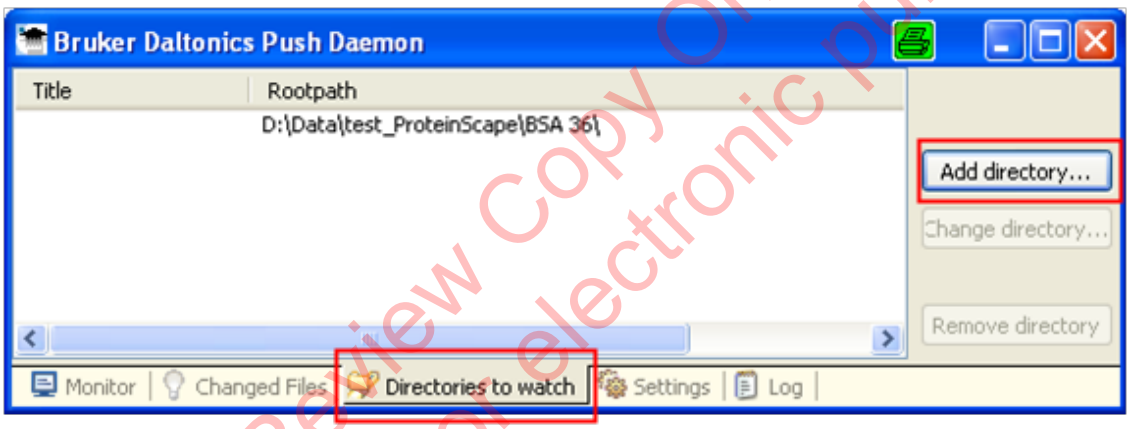

Figure 4-8 Directories to watch page of the Bruker Daltonics Push Daemon

4. Select the desired directory by clicking the open dialog box button ... in the **Edit watch directory** dialog box and click **OK**.

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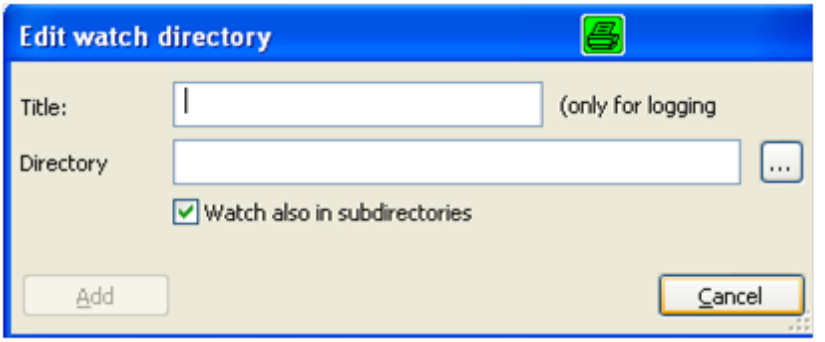

Figure 4-9 Edit watch directory dialog

The path to the directory will appear in the **Directory** field of the Edit watch directory dialog box.

a. Check the **Watch also in subdirectories** option to include subdirectories of the chosen directory.
b. Type a **Title** (optional, used for logging purposes) and click **Add**. The chosen directory is added to the list of watched directories.

5. To change the **Title** or **Rootpath** of a watched directory, select it in the list and click **Change directory**. To remove a watched directory select it and click **Remove directory**.
6. Click the **Settings** tab, select **Automatic Upload Mode** and enter `[server name]:8080` into the **Server Name or IP Address** field. Check the **Login to ProteinScape** box and enter your ProteinScape server username into the **User** field and click **Apply**.

Configuration is complete.

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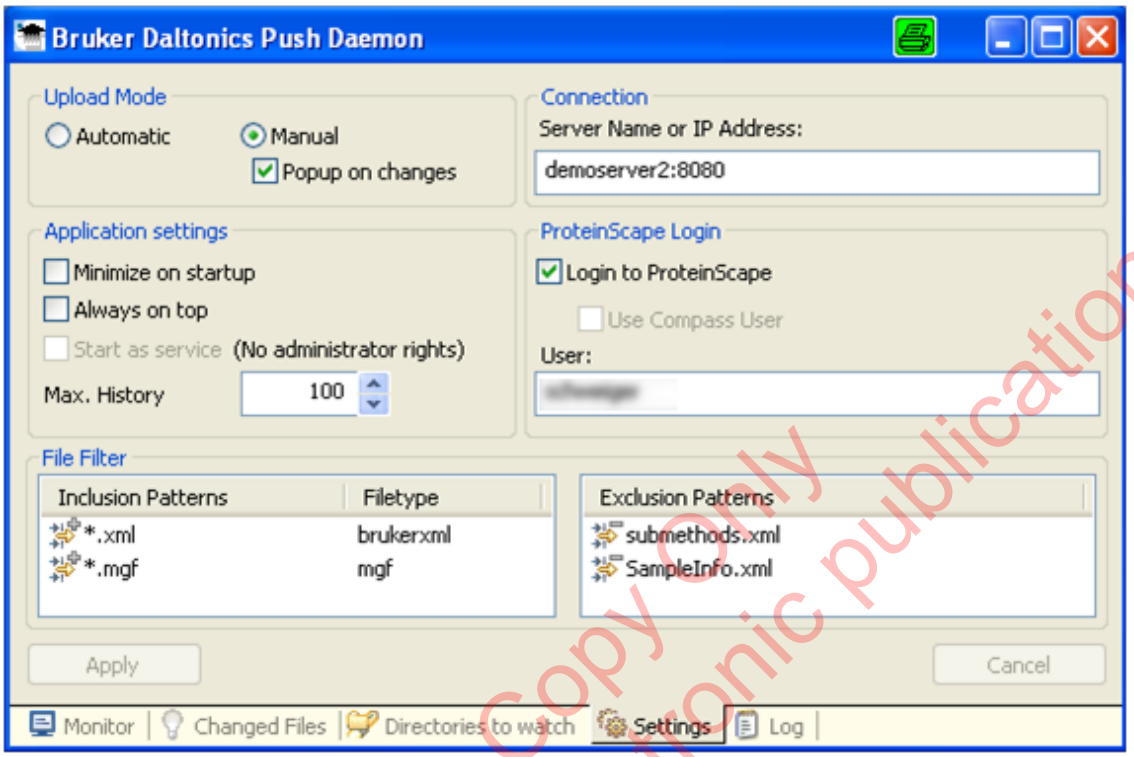

Figure 4-10 Settings page of the Bruker Daltonics Push Daemon

4.1.2.3 Starting Automatic Import of ESI Data

If the Push Daemon is active and has been configured for automatic transfer (see section 4.1.2.2), ESI data import will start automatically when a new peak list is saved in a watched directory.

4.1.3 Transferring MALDI MS Data into ProteinScape

MALDI MS data can be transferred into ProteinScape as single MALDI spectra (using flexAnalysis) or as complete LC-MALDI runs (using WARP-LC).

4.1.3.1 Transferring Single MALDI Spectra using flexAnalysis

Single MALDI spectra can be transferred from flexAnalysis (FA) to ProteinScape using **Automatic** or **MALDI Target** mode.

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Note The psClient ActiveX module must be installed on the FA computer. This module is part of the complete ProteinScape client installation.

A single MS or MS/MS spectrum that is open in FA can be transferred to ProteinScape by selecting **Tools > ProteinScape > Send Selected Spectrum**.

Transferring Spectra using Automatic Mode

Automatic mode is used in automation contexts, for example, for assigning spectra from MALDI target positions that have been measured in an AutoXecute run to spots of a 2D gel.

The node (for example, Gel Spot) in ProteinScape to which a spectrum will be exported can be defined when setting up an AutoXecute run. The mapping of MALDI target positions to nodes can be adapted by changing the order of the nodes.

Such spectra are automatically flagged for ProteinScape export and the FA **Automatic** mode tab displays the destinations of the respective spectra. Spectra will be automatically exported to the relevant nodes once the measurement is complete.

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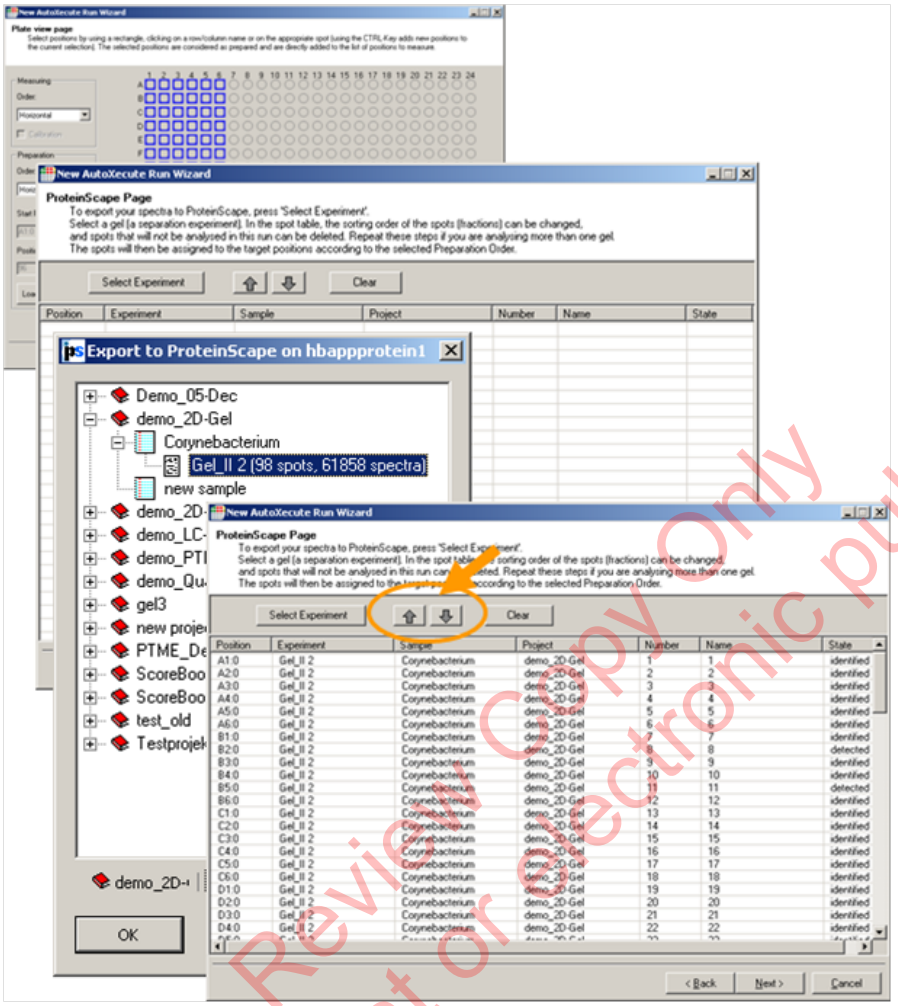
New AutoXecute Run Wizard
ProteinScape Page
To export your spectra to ProteinScape, press 'Select Experiment'.
Select a gel (a separation experiment). In the spot table, the sorting order of the spots (fractions) can be changed,
and spots that will not be analysed in this run can be deleted. Repeat these steps if you are analysing more than one gel.
The spots will then be assigned to the target positions according to the selected Preparation Order.
Select Experiment
Clear
Position
Experiment
Sample
Project
Number
Name
State
Export to ProteinScape on hbappprotein1
Demo_05-Dec
demo_2D-Gel
Corynebacterium
Gel_II 2 (98 spots, 61858 spectra)
new sample
gel3
test_old
OK
< Back
Next >
Cancel

Figure 4-11 Defining ProteinScape export information in AutoXecute

Transferring Spectra using MALDI Target Mode

Transferring spectra in **MALDI Target** mode creates a (MALDI) **Target** node in the Project Navigator. The location (parent node) and the name of the MALDI Target must be specified.

Target position sub-nodes are created that correspond to the target positions of the spectra. Selected spectra are added sequentially to Target nodes (A1 followed by A2, A3 and so on).

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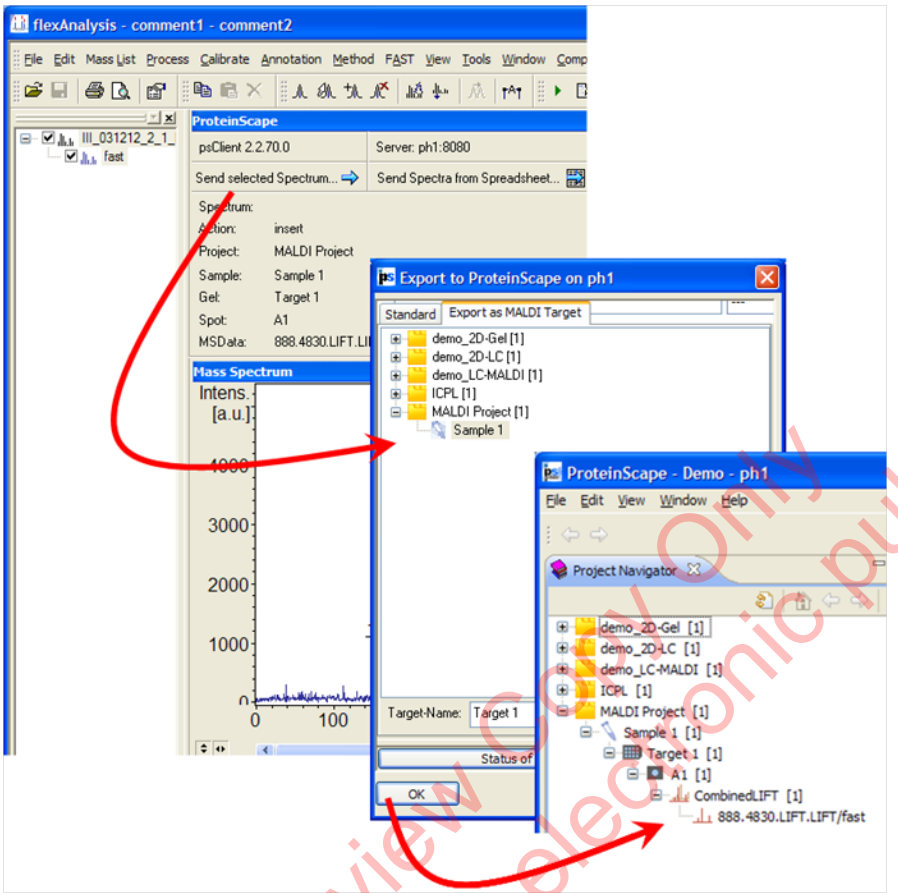

Figure 4-12 **Transferring MALDI spectra to ProteinScape using flexAnalysis MALDI Target mode**

Transferring Data from flexAnalysis to ProteinScape using Automatic Mode

This mode is only valid for the gel workflow in an automation context, that is, for assigning spectra to spots of a 2D gel that have been measured in an AutoXecute run. The Gel Spot node in ProteinScape to which a spectrum will be exported can be specified when setting up an AutoXecute run.

Selecting the **Show ProteinScape features in wizard** check box enables users to select the Experiment ID.

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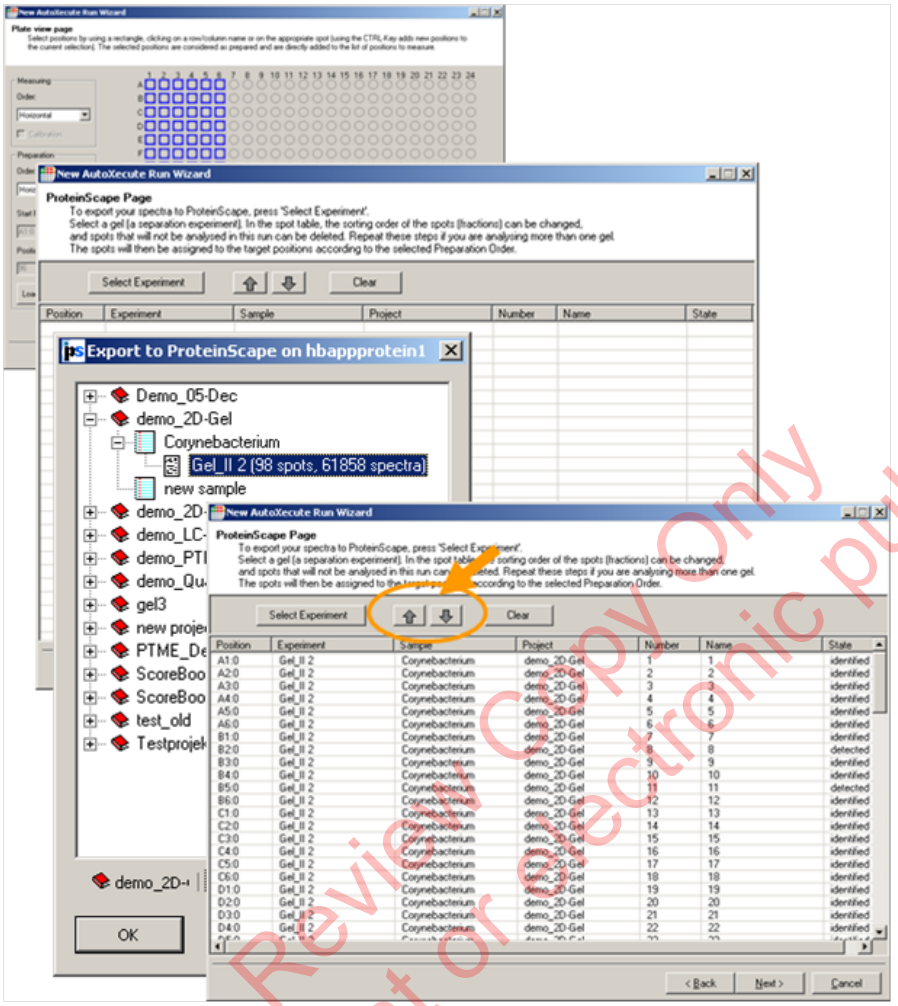
New AutoXecute Run Wizard
ProteinScape Page
To export your spectra to ProteinScape, press 'Select Experiment'.
Select a gel (a separation experiment). In the spot table, the sorting order of the spots (fractions) can be changed,
and spots that will not be analysed in this run can be deleted. Repeat these steps if you are analysing more than one gel.
The spots will then be assigned to the target positions according to the selected Preparation Order.
Select Experiment
Clear
Position
Experiment
Sample
Project
Number
Name
State
Export to ProteinScape on hbappprotein1
Demo_05-Dec
demo_2D-Gel
Corynebacterium
Gel_II 2 (98 spots, 61858 spectra)
new sample
gel3
test_old
OK
< Back
Next >
Cancel

Figure 4-13 Transferring data from flexAnalysis to ProteinScape using Automatic mode

In such cases, the spectra are automatically flagged for ProteinScape export and the FA **Automatic** mode tab displays the destinations of the respective spectra. The spectra will be sent to the spots of a 2D gel. The spots are displayed on the ProteinScape page of the **New AutoXecute Run** wizard along with the MALDI target positions. The mapping of positions to spots can be adapted by changing the order of the spots. Having done this, the spectra will be automatically exported to the relevant spots once the measurement has been made.

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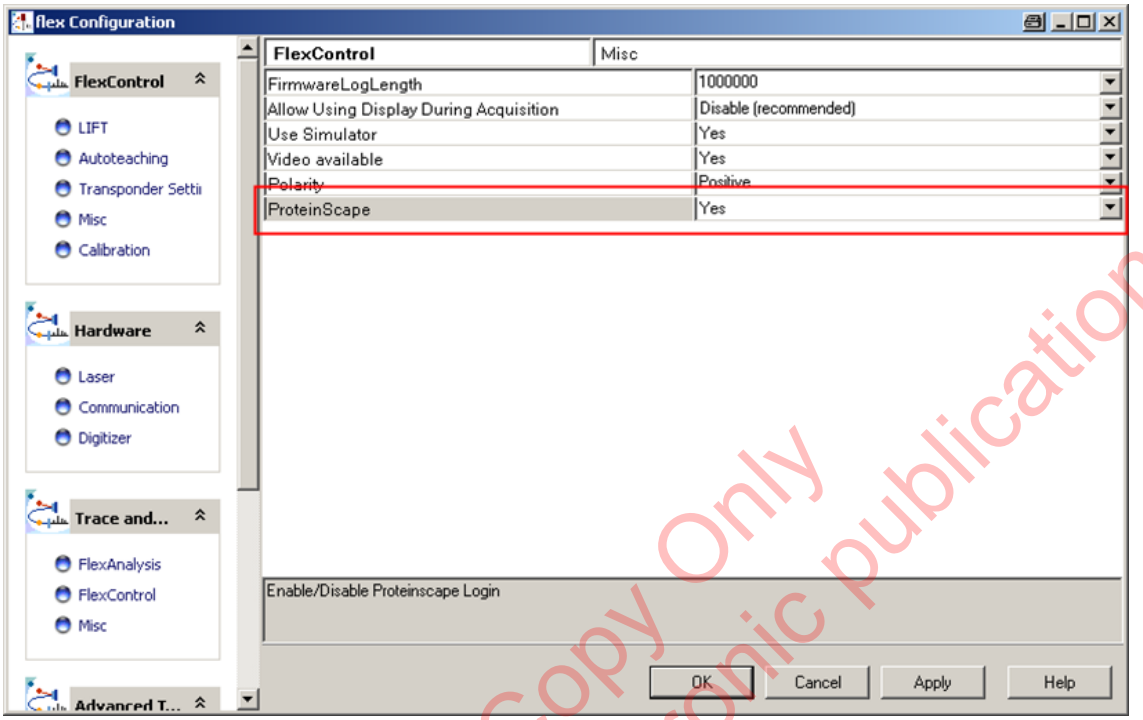

Figure 4-14 flex Configuration dialog

Opening the flex Configuration dialog and selecting the **Yes** option under **FlexControl > Misc > Proteinscape** means that all Bruker Software (flexControl, all flexAnalysis processes and WARP-LC need only login once into ProteinScape.

Transferring Data from flexAnalysis to ProteinScape using MALDI Target Mode

If spectra are exported in MALDI Target Mode a MALDI Target entry will be generated in ProteinScape. The location and the name of the MALDI Target must be specified.

Target positions will be generated that correspond to the target positions of the spectra. If there is already a MALDI Target with this name, the spectra will be added to this target.

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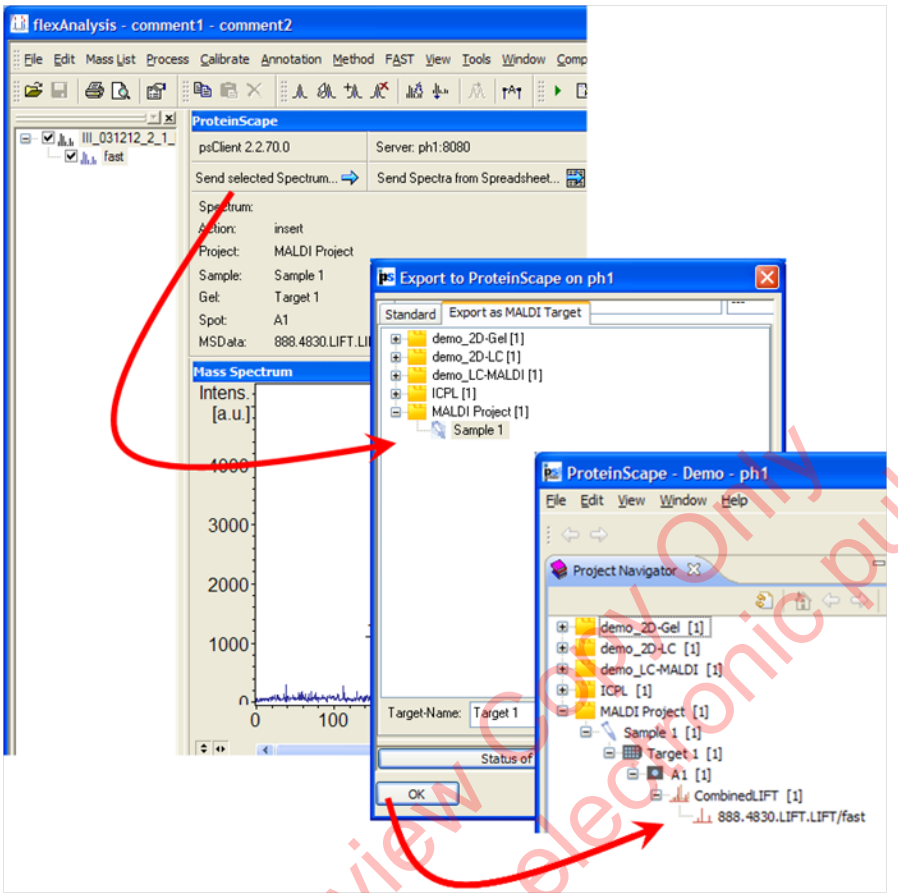

Figure 4-15 Transferring data from flexAnalysis to ProteinScape using MALDI Target mode

4.1.3.2 Transferring LC-MALDI Runs using WARP-LC

After acquisition and Compound List calculation, LC-MALDI run data sets are transferred to ProteinScape using WARP-LC.

►► To transfer LC-MALDI run data sets to ProteinScape

1. In WARP-LC, click **Send Data to > ProteinScape**.

   a. Select the **Start Search** check box to automatically trigger a protein search when data transfer is complete.

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b. Select the **Include compounds lacking MS/MS data** check box to transfer all MS spectra, even if there are no associated MS/MS spectra. Select this option if full MS spectra (not just precursor masses) of the compounds are required.To speed up data transfer,this option is cleared by default.

The **Export to ProteinScape** dialog box will open displaying **Project** and **Sample** nodes in the Project Navigator.

2. Select a destination node (usually a **Sample** node) for the compounds data and click **OK**.

The LC-MALDI run data is exported to the selected node. A progress bar indicates the progress of data transfer. When data transfer is complete, a search is automatically triggered.

In Stable Isotopic Label Experiments (SILE), the search results are automatically sent back to WARP-LC for calculation of peptide ratios. These results are in turn automatically transferred back to ProteinScape (see section 7.1.6).

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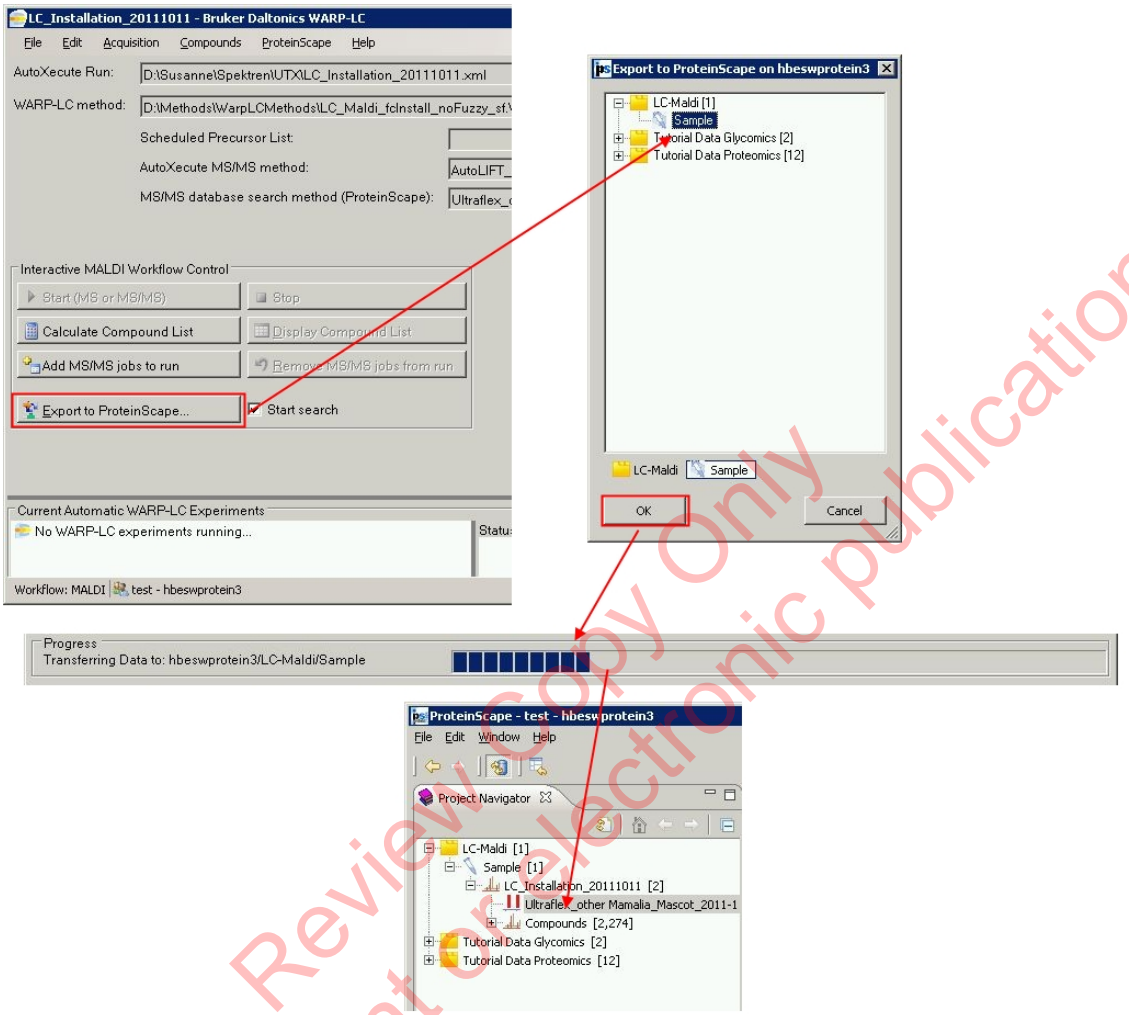

Figure 4-16 Transferring an LC-MALDI run to ProteinScape using WARP-LC

4.1.4 Defining the Raw Data Path

The ProteinScape database stores MS data on a peak list level. However, ProteinScape also stores the path to the raw data. This path is saved when a spectrum is transferred to ProteinScape and is displayed in the **Source** field of the MS or LC-MS/MS data set Main View.

Note that the validity of this path depends on the client computer.

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For example, if a data set was saved under `d:\data\my_data sets` on the acquisition PC, and the user uses a different PC as ProteinScape client for an in-depth analysis, the data will not be found under `d:\data\my_data sets` on this computer.

The data might instead be accessible either on a mapped network drive, for example, `p:\data\my_data sets`, or perhaps on a UNC network path, for example, `\\acquisition_pc\data\my_data sets`.

To ensure that a data set can be found on the same path on every client computer, the `d:\` drive of the acquisition PC can be locally mapped to a virtual drive `p:\` where data can be stored for example, under `p:\data`.

Other computers can map the `d:\` drive of the acquisition PC as `p:\`, and the data are visible in the same location.

4.1.4.1 LC-MS Survey View

Clicking a **Combined Data** node in the **Project Navigator** opens the **LC-MS Survey View**. This View shows the peaks / compounds that are stored in the ProteinScape database on the raw data path.

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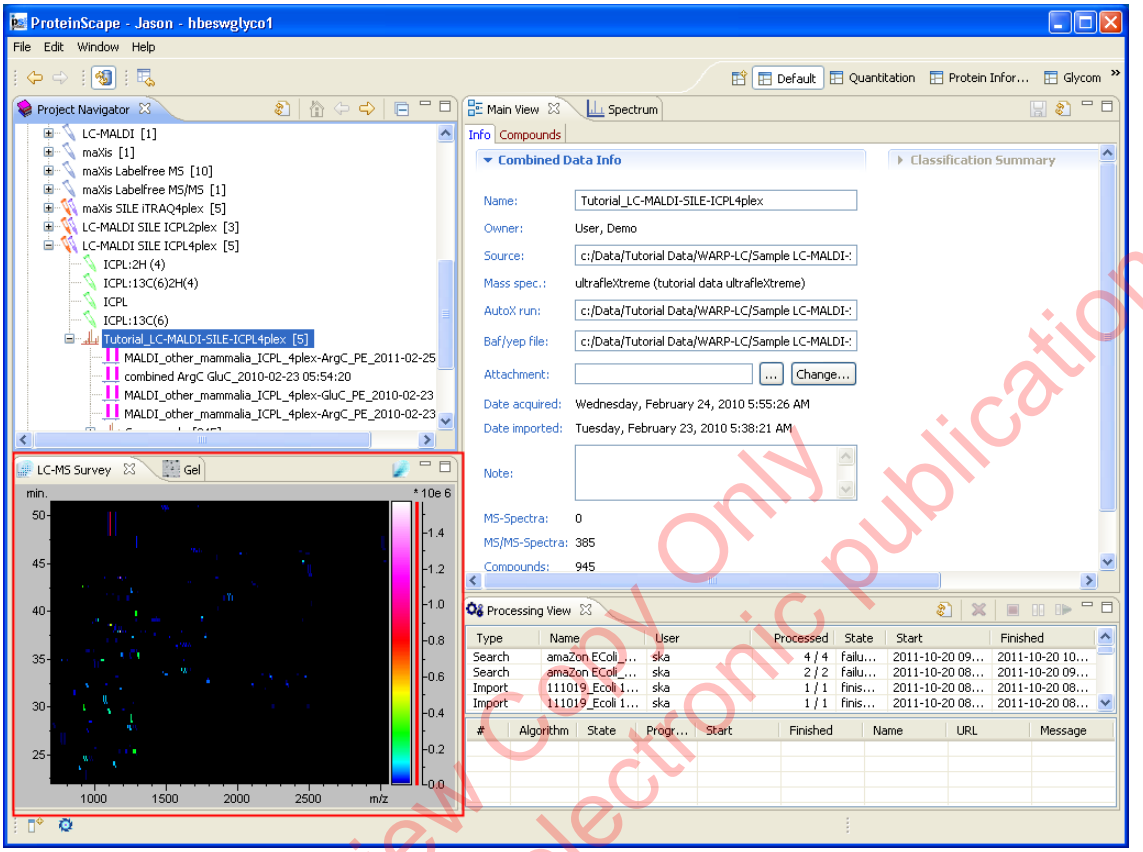

Figure 4-17 LC-MS Survey View

If the raw data path is not valid, a dialog box opens to assist users in finding the correct location on the hard disk or in the network. The path to the folder selected using this dialog box is stored as the new raw data path, and the raw data are loaded into the **LC-MS Survey View**.

4.1.4.2 Manually Changing the Raw Data Path

The raw data path of single MS spectra or LC-MS/MS runs is found in the respective Data Info area of the Main View.

To change the raw data path, edit the **Source**field and click the **Save** button in the pane toolbar (see Figure 4-18).

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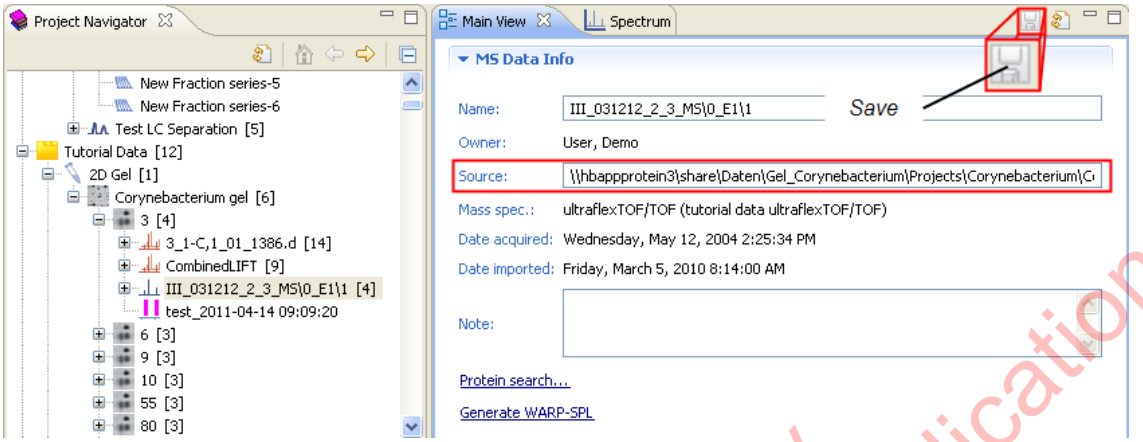

Figure 4-18 Manually changing and saving the raw data path

4.1.4.3 Adjusting the Raw Data Path of Multiple Data Sets

Sometimes it is necessary to move raw data files to another location, for example a portable hard disk or another computer.

In such cases the raw data paths stored in the ProteinScape database must be adjusted accordingly (see Figure 4-19).

►► To adjust the raw data paths of multiple data sets

1. Right-click the ProteinScape node with which the raw data is associated and select **Adjust Raw Data Paths** from the menu.

   The **Raw Data Path Adjustment** dialog box opens.

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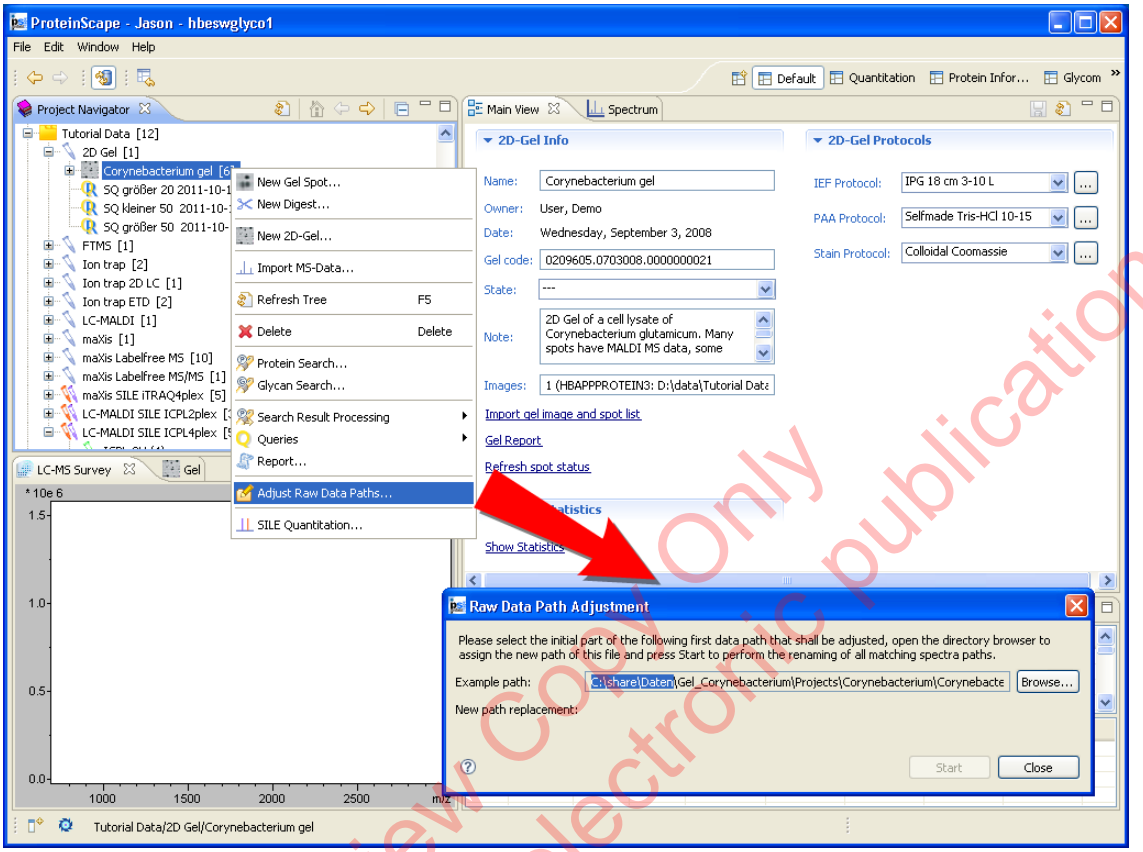

Figure 4-19 Adjusting raw data paths of multiple data sets

2. Highlight the section of the **Example path** to be adjusted. The selected section must begin at the root directory and end just before or after a separator.
3. Click the **Browse** button and go to the new location of the raw data.
4. Click **OK**.

   The **New path replacement** field will display the new raw data path. The raw data paths of data sets in the selected node and all its sub-nodes will be adjusted accordingly.

5. Click **Close**.

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4.2 Viewing Combined Data Sets

LC-MS/MS data are acquired as one data set, and one peak list is exported. Data can be Viewed in the **LC-MS Survey** View (see section 6.4.4).

The **Combined Data Info** area on the **Info** tab of the data set's Main View displays information about the combined MS/MS data set (for example, an LC-MS/MS run).

The **Compounds** tab of the data set's Main View displays compounds in the data set.

Identified peptides and glycans in a combined data set can be displayed in separate tables by selecting and right-clicking compounds in the **Compounds** tab and selecting **Show > Identified Peptides/Glycans**.

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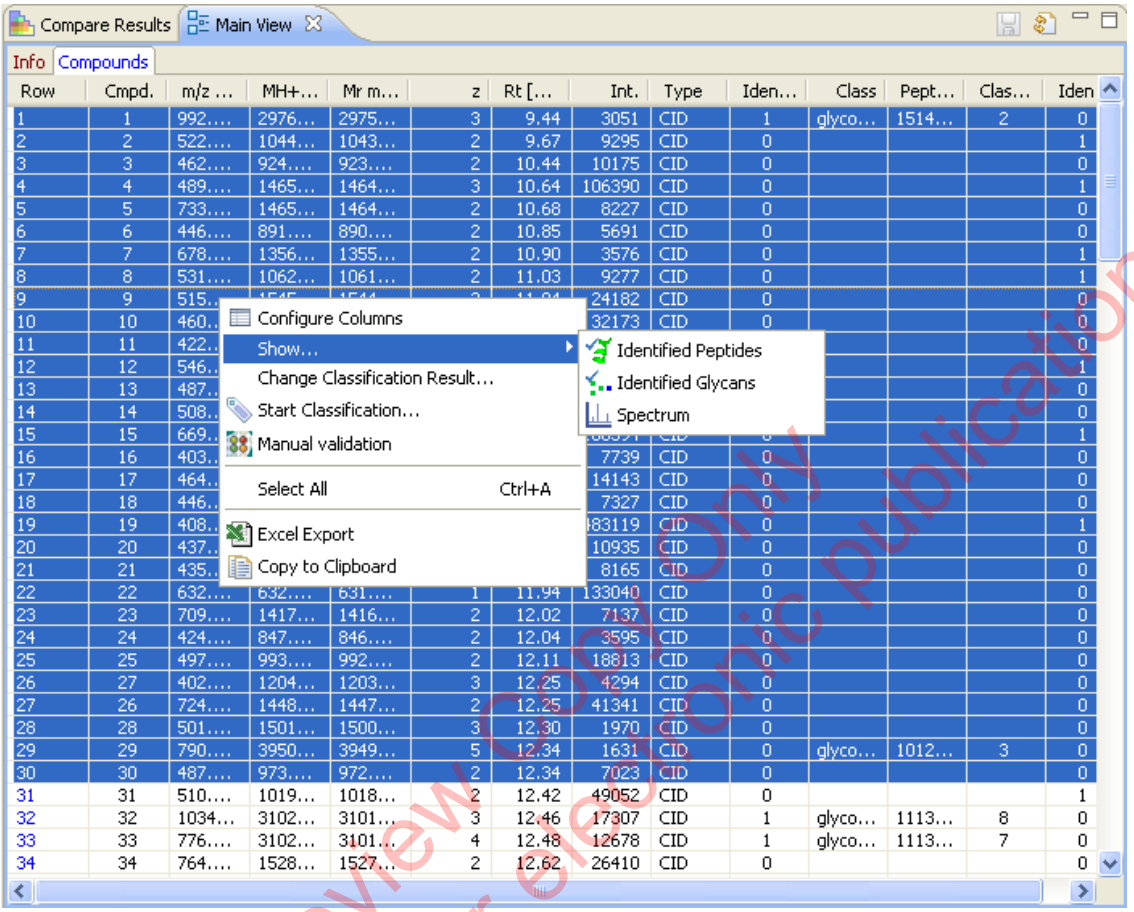

Row	Cmpd.	m/z ...	MH+...	Mr m...	z	Rt [...	Int.	Type	Iden...	Class	Pept...	Clas...	Iden
1	1	992....	2976...	2975...	3	9.44	3051	CID	1	glyco...	1514...	2	0
2	2	522....	1044...	1043...	2	9.67	9295	CID	0				1
3	3	462....	924....	923....	2	10.44	10175	CID	0				0
4	4	489....	1465...	1464...	3	10.64	106390	CID	0				1
5	5	733....	1465...	1464...	2	10.68	8227	CID	0				0
6	6	446....	891....	890....	2	10.85	5691	CID	0				0
7	7	678....	1356...	1355...	2	10.90	3576	CID	0				1
8	8	531....	1062...	1061...	2	11.03	9277	CID	0				1
9	9	515..					24182	CID	0				0
10	10	460..					32173	CID	0				0
11	11	422..											0
12	12	546..											1
13	13	487..											0
14	14	508..											0
15	15	669..											1
16	16	403..					7739	CID	0				0
17	17	464..					14143	CID	0				0
18	18	446..					7327	CID	0				0
19	19	408..					83119	CID	0				1
20	20	437..					10935	CID	0				0
21	21	435..					8165	CID	0				0
22	22	632....	632....	631....	1	11.94	133040	CID	0				0
23	23	709....	1417...	1416...	2	12.02	7137	CID	0				0
24	24	424....	847....	846....	2	12.04	3595	CID	0				0
25	25	497....	993....	992....	2	12.11	18813	CID	0				0
26	27	402....	1204...	1203...	3	12.25	4294	CID	0				0
27	26	724....	1448...	1447...	2	12.25	41341	CID	0				0
28	28	501....	1501...	1500...	3	12.30	1970	CID	0				0
29	29	790....	3950...	3949...	5	12.34	1631	CID	0	glyco...	1012...	3	0
30	30	487....	973....	972....	2	12.34	7023	CID	0				0
31	31	510....	1019...	1018...	2	12.42	49052	CID	0				1
32	32	1034...	3102...	3101...	3	12.46	17307	CID	1	glyco...	1113...	8	0
33	33	776....	3102...	3101...	4	12.48	12678	CID	1	glyco...	1113...	7	0
34	34	764....	1528...	1527...	2	12.62	26410	CID	0				0

Figure 4-20 Compounds table shortcut menu

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Multiple MS/MS Spectra

MS/MS spectra are always collected within a Combined Data node. A protein search cannot be performed on a single MS/MS spectrum; it must be performed on the Combined Data node that contains the combined MS/MS data set.

Table 4-1 Combined Data Set Main View parameters

Parameter	Comments
Name	Name of the spectrum.
Owner	The user who imported or exported the spectrum to ProteinScape.
Source	The complete path to the spectrum, usually to the file `1r` or `peaklist.xml`. This is the path that will be transferred to BioTools. If the raw data is moved or the spectrum has been imported into ProteinScape from another PC, this path must be changed manually. BioTools can only access the raw data if this path is correct.
Mass Spec	The name of the MS instrument specified either in the raw data or manually during the interactive MS data import.
AutoX run / Baf/yep file	Depending on the MS instrument type.
Attachment	The complete path to an attached file (for example, a DataAnalysis report).
Date acquired/ imported	The acquisition date specified in the raw data and the date of import into ProteinScape.
Note	Free text field for additional information.
MS-Spectra	Number of MS spectra in the data set.
MS/MS-Spectra	Number of MS/MS spectra in the data set.
Compounds	Number of compounds in the data set.
Protein Search	Button to start the protein search.

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5 Identifying Proteins

ProteinScape performs the following steps to identify proteins:

- Peptide identification
- Protein list compilation (MS/MS searches only)
- Protein assessment
- ID refinement

The parameters used for the first three steps are defined in the respective pages of the **Protein Searches** dialog (see section 5.3). Options for ID refinement include repeating searches with altered parameters, second-round searches and decoy strategies.

5.1 Performing Protein MS/MS Searches

MS/MS searches are launched from combined MS/MS (PFF) data sets ( ).

5.2 Selecting the Data used for Protein MS/MS Searches

Protein MS/MS searches can be launched from nodes containing data sets or the data set nodes themselves. Launching a search from a non-data node (Project, Sample, LC, Gel, or Digest) enables the user to select and filter the data sets used for the search.

►► To select and filter data used for protein searches (launch search from a non-data node)

1. Right-click a non-data node (Project, Sample, LC, Gel, or Digest) containing the desired data set(s) and select **Protein Search** from the shortcut menu.

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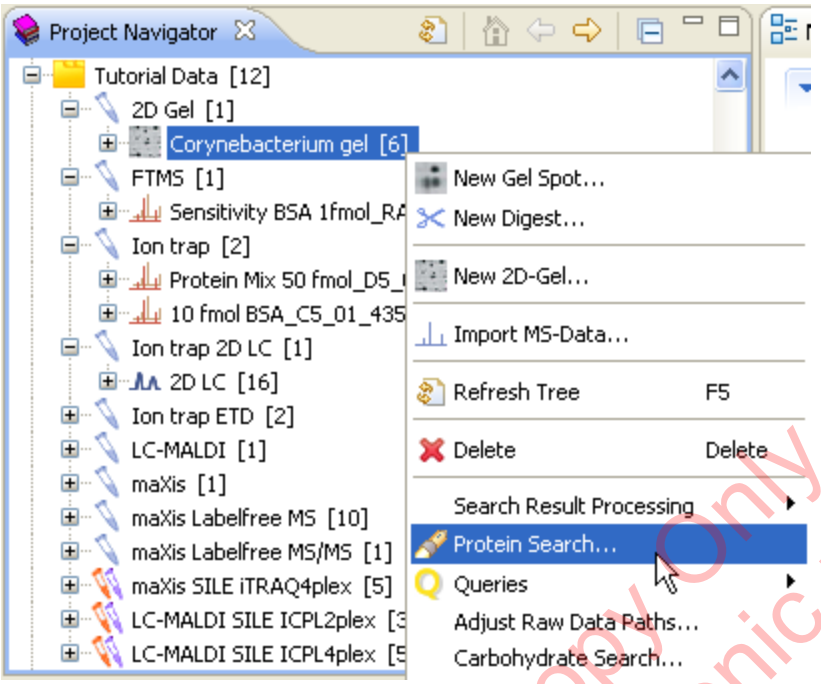

Figure 5-1 Launching a protein search from a 2D Gel node in the Project Navigator

The **Protein Searches > Spectra Selection** dialog box opens.

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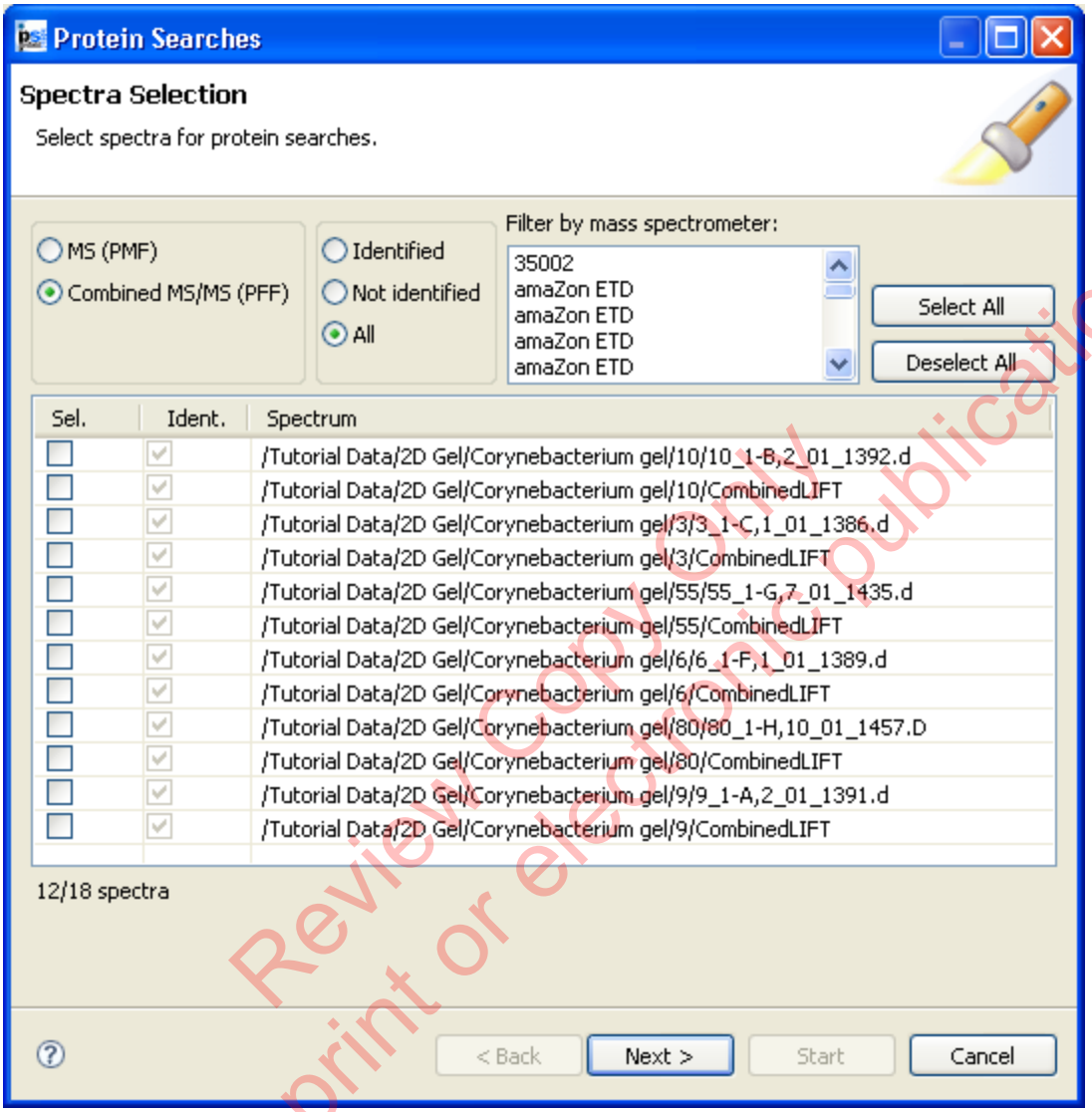

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Figure 5-2 Protein Searches > Spectra Selection dialog

2. Select the data type **Combined MS/MS (PFF)** and identification status (**Identified**, **Not identified** or **All**).

   Selecting **Combined MS/MS (PFF)** will list all combined data sets in the node.

   The list of data sets can be filtered by selecting one or more mass spectrometers from the **Filter by mass spectrometer** list.

3. In the **Sel.** column, select the box next to the data that will be used for the protein search.

   Use **Select All** or **Deselect All** to include or remove all data sets from the selection.

4. Click **Next**.

   The **Protein Searches** dialog opens (see section 5.3).

►► To launch a protein search directly from an MS/MS data node

- Right-click a Combined Data ( ) node and select **Protein Search** from the shortcut menu.

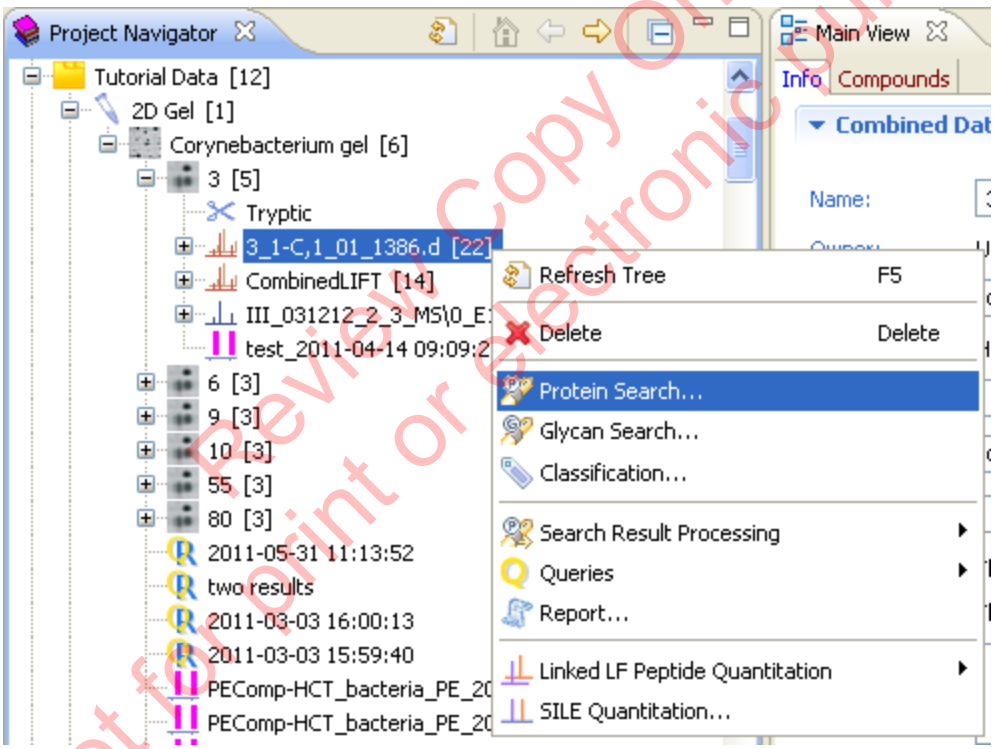

Figure 5-3 Launching a protein search from a Combined Data node in the Project Navigator

The **Protein Searches** dialog opens (see section 5.3). The type of data (MS/MS [PFF]) is indicated in parentheses in the dialog header.

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5.3 Protein Searches Dialog

The **Protein Searches** dialog is a multipage dialog used to define parameters for

- peptide identification,
- protein list compilation (MS/MS data only) and
- protein and peptide assessment.

5.3.1 Protein Search Parameters

5.3.1.1 General Settings

The following parameters are set in the **General Settings** area of the **Protein Searches > Search Parameter Configuration** dialog.

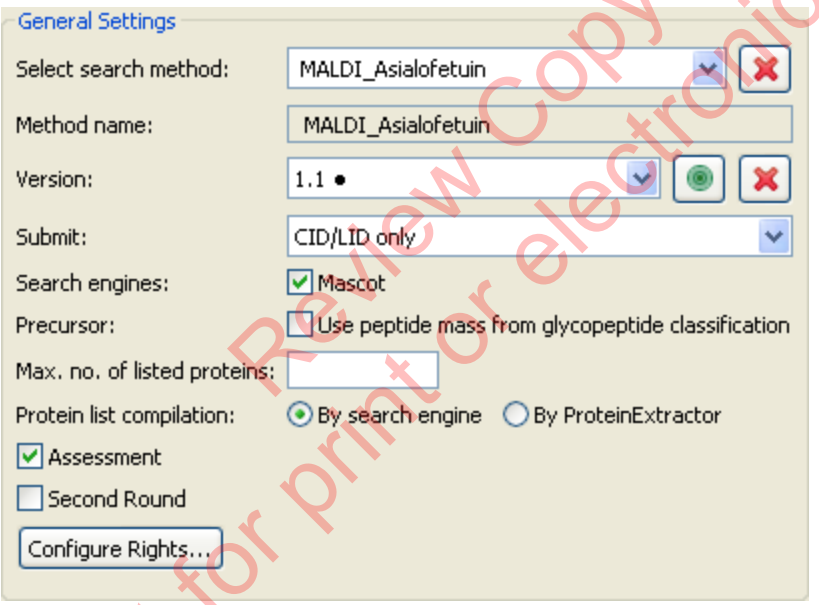

Figure 5-4 General Settings area of the Protein Searches Parameter Configuration window

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Table 5-1 General Settings parameters

Parameter	Comments
Select search method	Select an existing search method from the drop-down list. To delete a method, select it in the list and click .
Method name	Displays the selected search method.
Version	Select a version using the drop-down list. Click to define the selected method version as the default version. To delete a version, select it in the list and click .
Submit	Select to submit **All spectra**, **CID/LID** only, or **ETD/ECD** only.
Search engines	Select the relevant box(es) to select the desired search engine(s).
Precursor: Use peptide mass…	The peptide mass determined by a classification or manually entered in the compounds table will be used as a virtual precursor in the protein search.
Max. no of listed proteins	Limit the total number of proteins listed. Must be an integer.
Protein list compilation	Compile protein list using the search engine (see section 5.3.4.1) or the ProteinScape ProteinExtractor (see section 5.3.4.2). Parameters for search engines are set in their respective tabs.
Assessment	Perform automatic protein and peptide assessment (see section 5.3.5).
Second round	Perform second round searches (see section 5.3.6).
Configure Rights	Launch the **Configure Method Access** window.

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5.3.2 Protein Search Methods

A protein search method defines the parameters used to search for peptide matches (and as a result protein matches) in ProteinScape.

The protein search method defines the search engine used, modifications that should by taken into account, the charge on peptides, mass tolerances, and enables filtering of results according to peptide or protein score.

5.3.2.1 Using Existing Protein Search Methods

►► To use an existing protein search method for a protein search

1. Select an existing search method from the **Select search method** drop-down list.
2. Select the desired method version from the **Version** drop-down list.
3. Click **Start** to start the protein search.

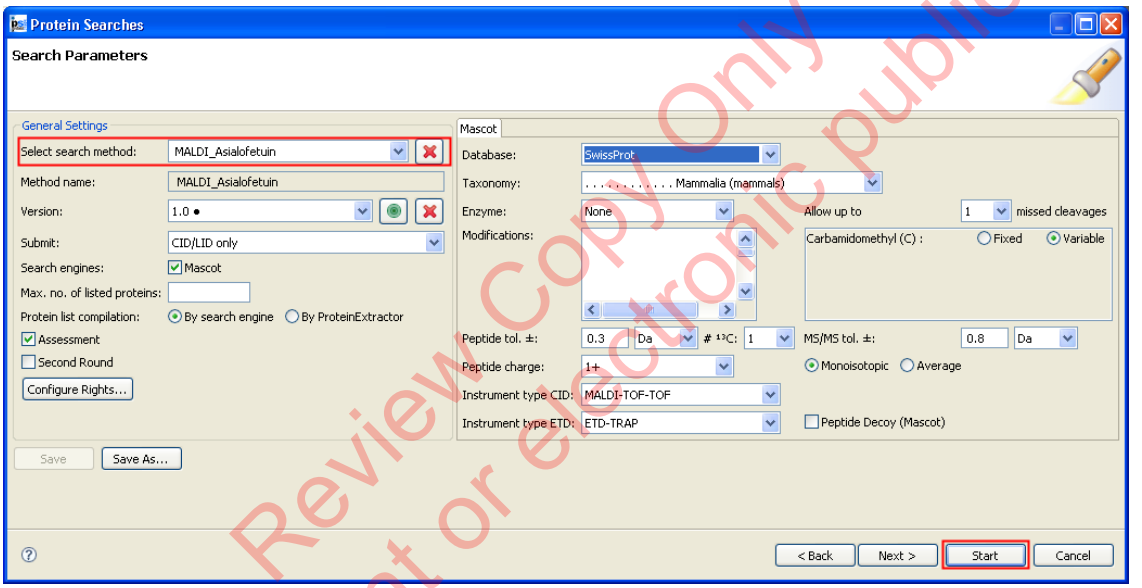

Figure 5-5 Protein Searches > Search Parameters dialog

►► To use the most recently used protein search method for a protein search

1. Select **Repeat Protein Search** in the shortcut menu of an existing Search Result node, or **Repeat Search** in the **Info > Search Result Info** area of its Main View.

   The method used for the most recent search is opened.

2. Select the desired method version from the **Version** drop-down list.
3. Click **Start** to start the protein search.

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5.3.2.2 Editing and Creating Protein Search Methods

Protein search methods cannot be changed after they are created and saved to the ProteinScape database. Any changes to an existing method can be saved as an updated version of the existing method or as a new method.

The effect of changes to an existing method can be investigated by editing the method and starting the protein search before saving the method. This creates a "temporary" search method.

Note The **Repeat Protein Search** function (see section 5.7) is not available in the Search Result node of searches performed using a temporary search method.

►► To create a new version of an existing protein search method

1. Select **Edit > Methods > Protein MS(MS/MS) Search** to open the **Protein Searches** dialog.

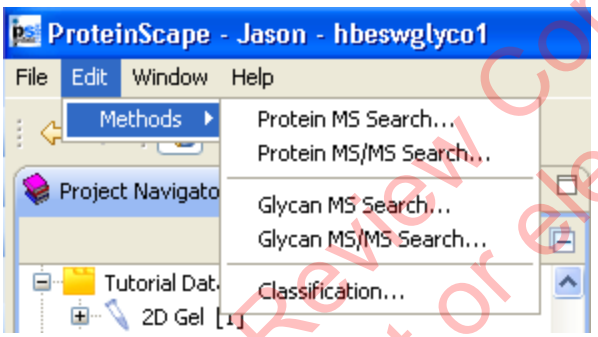

Figure 5-6 Edit > Methods menu

2. Select an existing search method from the **Select search method** drop-down list.
3. Make the desired changes to the search parameters, protein list configuration and assessment configuration in the **Protein Searches** dialog pages.
4. Click **Save** and type a new version name in the **Save Method** dialog.
5. Click **OK** to close the dialog.

   The most recently created version of a protein search method is automatically set as the standard (default) version in the **Version** drop-down list (indicated by a closed circle next to the version name).

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►► To create a new protein search method

1. Select **Edit > Methods > Protein MS(MS/MS) Search** to open the **Protein Searches** dialog.
2. Make the desired changes to the search parameters, protein list configuration and assessment configuration in the **Protein Searches** dialog pages.
3. Click **Save As** and type a method name and first version name in the **Save As New Method** dialog.
4. Click **OK** to close the dialog.

   The new protein search method is added to the **Select search method** drop-down list.

5.3.2.3 Assigning User Rights to Search Methods

Clicking **Configure Rights** in the **General Settings** area of the **Protein Searches Assessment Configuration** page enables user rights to be assigned to individual users or groups.

When creating a new method, the “owner” initially has unique access rights. The new method is unavailable to other users until access rights are assigned.

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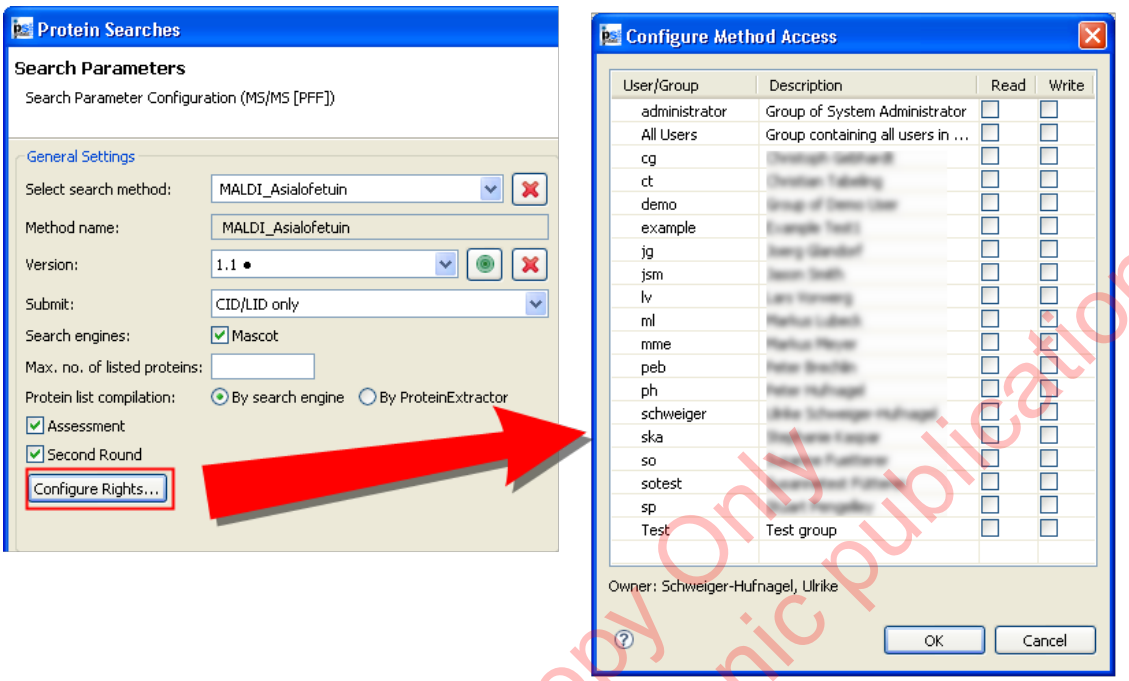

Figure 5-7 Configuring User Rights for protein search methods

5.3.3 Search Engines

The area to the right of the **General Settings** displays search engine parameters. Click the respective tab to access the parameter page for each search engine.

See the *ProteinScape Administrator's Manual* for information on how to introduce new parameters.

5.3.3.1 Mascot Parameters

The Mascot search engine and its parameters are described in detail at www.matrixscience.com.

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Table 5-2 Mascot search engine parameters

Parameter	Description
Modifications	Select desired modifications from the drop-down list. Press **CTRL** while clicking to select multiple modifications. Selected modifications are listed in the window to the right of the list. Use the buttons to assign them as **Fixed** or **Variable**.
Instrument type CID/ETD	Corresponds to **Instrument** in the Mascot parameter window. For LC MS/MS data with CID or ETD or CID and ETD spectra in one XML file, all types of abundant MS/MS spectra are searched with the appropriate instrument.
Peptide Decoy (Mascot)	Corresponds to the **Use Mascot** decoy option in the Mascot parameter window (see section 5.4).
Use Percolator	Select to use a post-search algorithm to recalculate peptide scores on the basis of a statistical analysis (Mascot 2.3 or later).

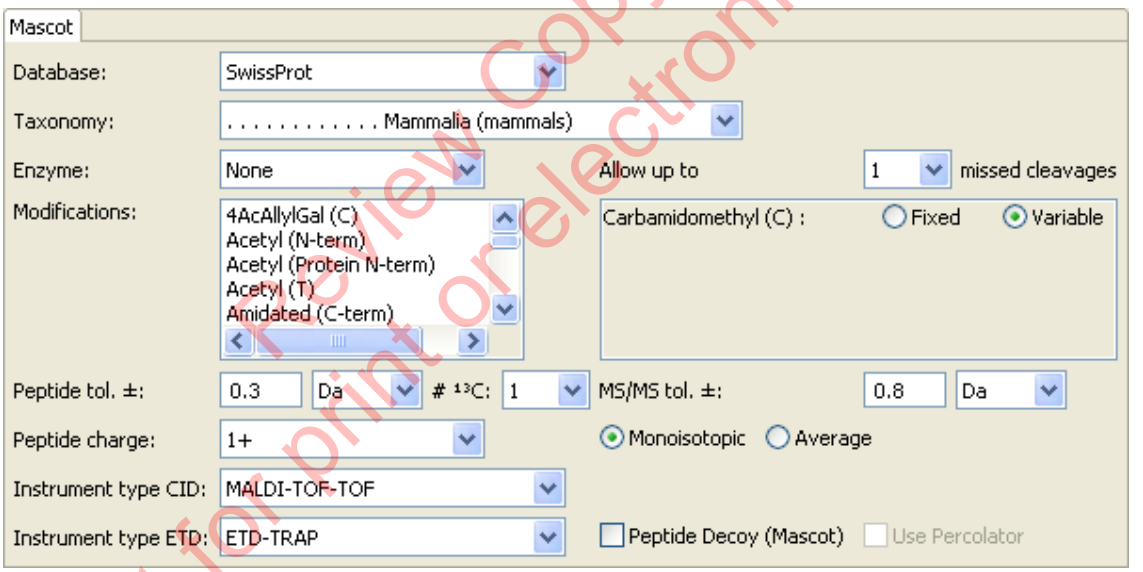

Figure 5-8 Search Parameter Configuration page

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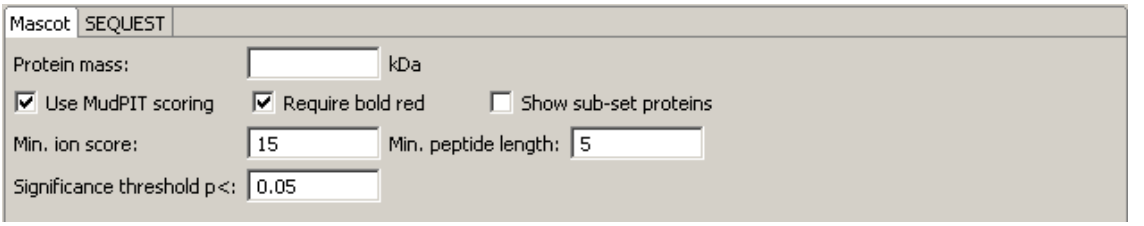

Figure 5-9 Protein List Compilation Configuration page

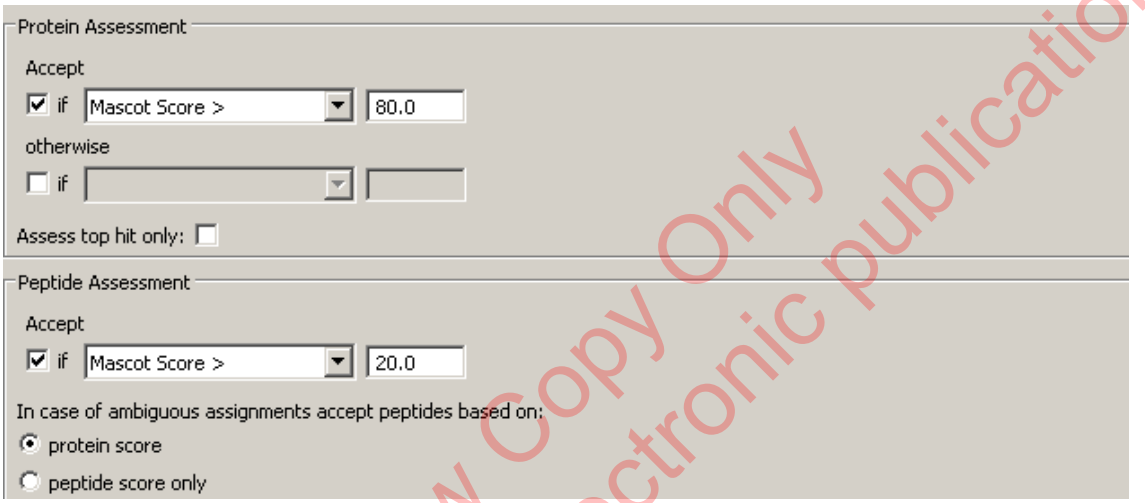

Figure 5-10 Assessment Configuration page

Note The parameters **#13C** and **Peptide Decoy** are not supported by Mascot versions earlier than 2.2. Although both parameters will be visible in the ProteinScape Mascot parameter window, they are ignored when the ProteinScape server is connected to a server running an earlier version of Mascot than 2.2.

Note The parameter **Use Percolator** is not supported by Mascot versions earlier than 2.3. Although the parameter will be visible in the ProteinScape Mascot parameter window, it is ignored when the ProteinScape server is connected to a server running an earlier version of Mascot than 2.3.

5.3.3.2 Phenyx Parameters

The Phenyx search engine and its parameters are described in detail at http://www.genebio.com/products/phenyx/. Modifications are entered in the same way as for Mascot searches.

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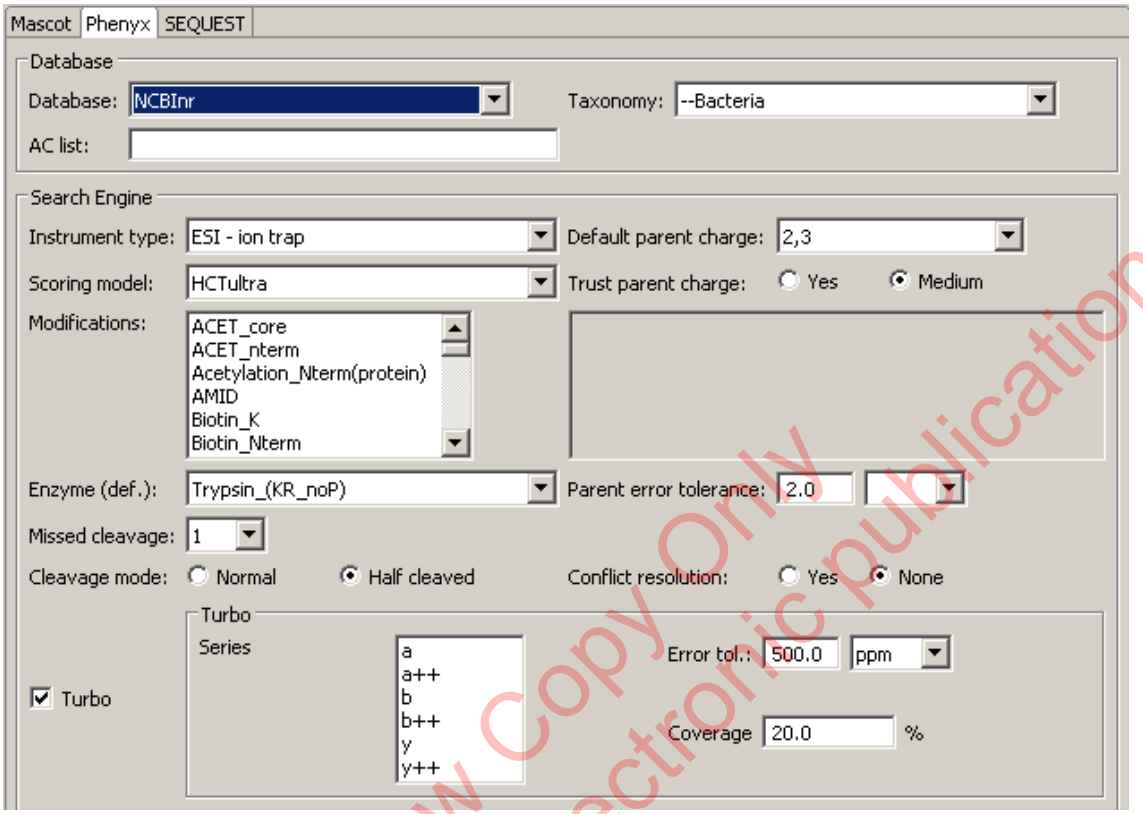

Figure 5-11 Search Parameter Configuration page (Phenyx)

Figure 5-12 Protein List Compilation Configuration page (Phenyx)

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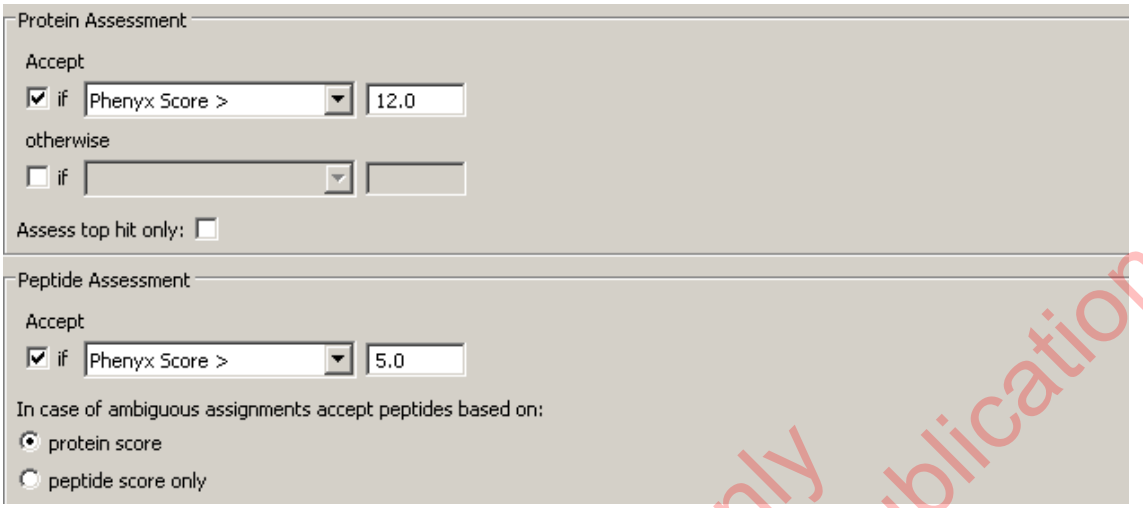

Figure 5-13 Assessment Configuration page (Phenyx)

5.3.3.3 SEQUEST Parameters

Modifications are entered in the same way as for Mascot searches.

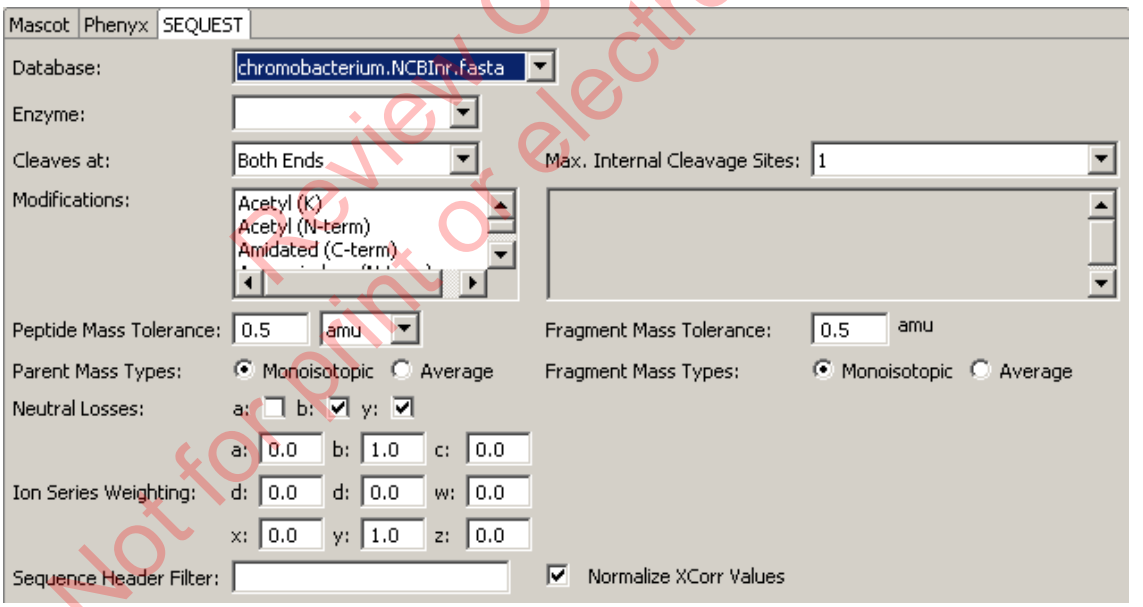

Figure 5-14 Search Parameter Configuration (SEQUEST)

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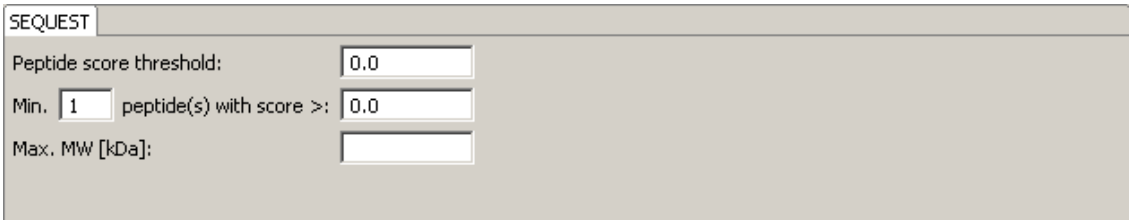

Figure 5-15 Protein List Compilation Configuration (SEQUEST)

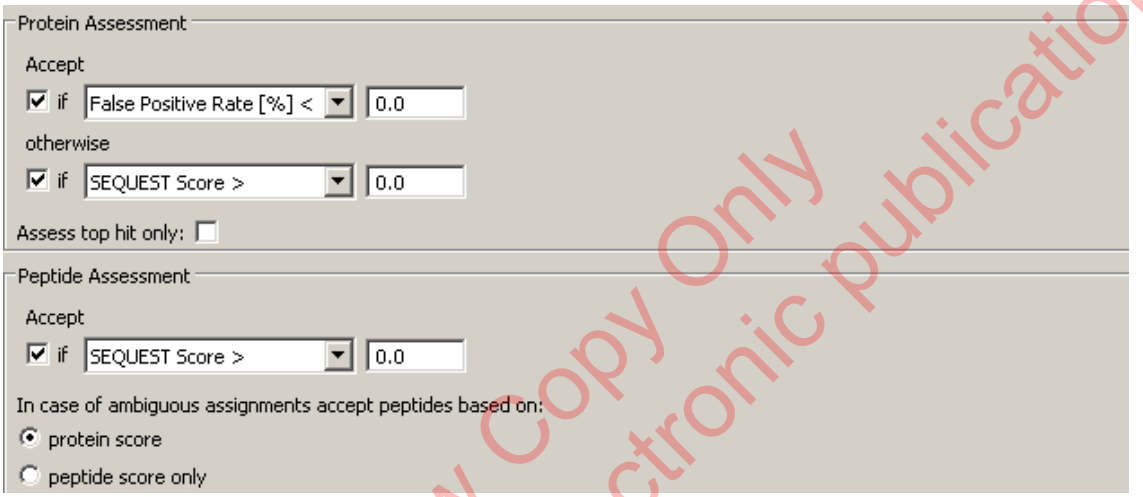

Figure 5-16 Assessment Configuration (SEQUEST)

5.3.4 Protein List Compilation

Protein lists can be compiled using individual search engines or the ProteinScape ProteinExtractor function.

Protein searches performed using ProteinExtractor are indicated by a pink search node symbol in the **Project Navigator** tree.

Protein searches that do not use ProteinExtractor are indicated by a red search node symbol .

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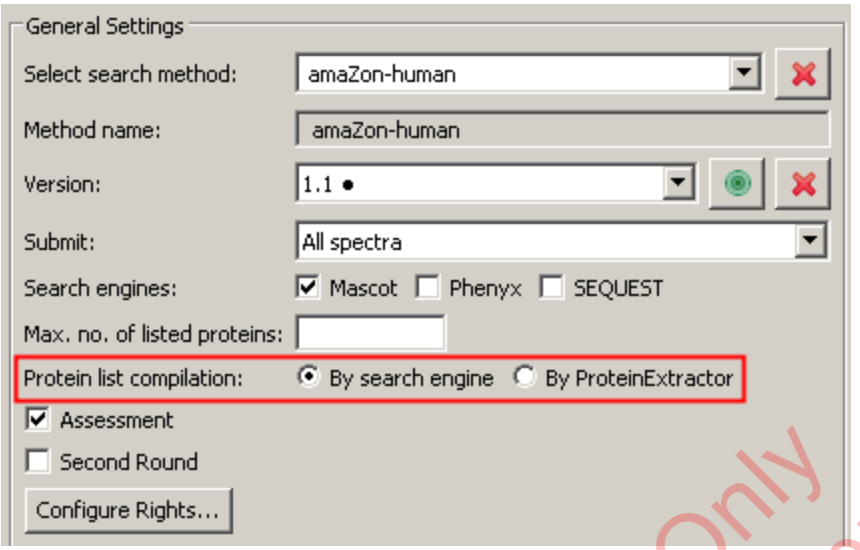

Figure 5-17 Defining the method used for protein list compilation

Protein list acceptance parameters are defined on the **Protein List Compilation Configuration** page of the **Protein Searches** dialog box. Refer to the respective search-engine literature for more information on parameters.

5.3.4.1 Compiling Protein Lists using a Search Engine

Open the Protein Searches dialog box, select the relevant search engine tab and click **Next >** to access the Protein List Compilation page.

Enter the relevant parameter settings as described in the respective search engine user manual.

5.3.4.2 Compiling Protein Lists using the ProteinExtractor

The ProteinScape ProteinExtractor feature can be used to compile protein lists from multiple search engines.

1. Select the desired search engine(s) and activate ProteinExtractor by selecting **By ProteinExtractor** button in the **General Settings > Protein list compilation** area of the **Protein Searches** dialog box (see section 5.3.1).
2. Click **Next >** to open the **ProteinExtractor Settings** dialog box.

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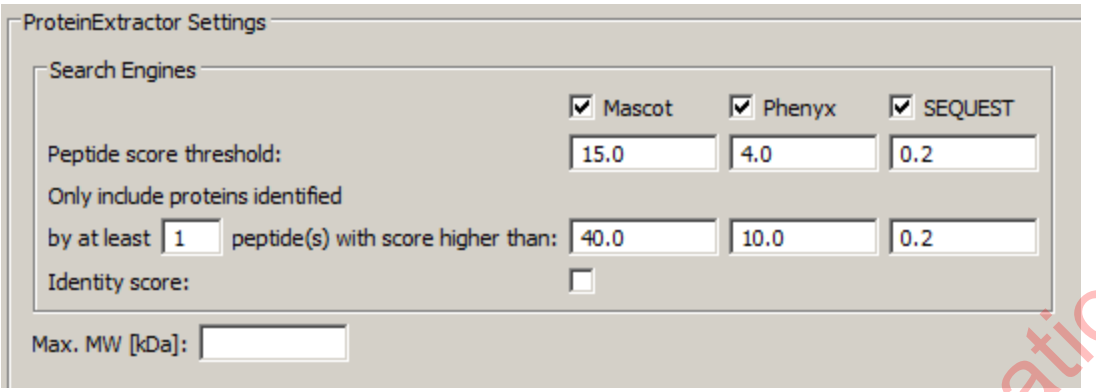

Figure 5-18 **ProteinExtractor Settings dialog**

Table 5-3 **ProteinExtractor Settings dialog parameters**

Parameter	Description
Search Engines	Select the search engine/s to apply to the peptide list.
Peptide score threshold	Only peptides with a peptide score above the value given for the respective search engine are included.
Only include proteins identified by at least … peptide(s) with score higher than:	All peptides above the **Peptide score threshold** are used for the protein score calculation. However, a protein appears in the result list only if the specified number of peptides meets this additional criterion. In the example above (see Figure 5-18), all proteins in the protein list must have been identified using at least one peptide with a Mascot score of > 40 or a Phenyx score > 10 or a Sequest score > 0.2.
Identity score	Specifies that a protein must have at least one peptide with a score above the **Identity Score** threshold calculated during a Mascot search. Refer to the Mascot manual for further details.
Max. MW [kDa]	Define the maximum molecular weight of proteins included in the list.

5.3.5 Protein and Peptide Assessment

The user can decide which of the proteins and peptides found in the search should be accepted.

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This can be done either manually or by selecting **Assessment** in the **General Settings** and defining suitable parameters in the **Protein-** and **Peptide Assessment** areas of the **Protein Searches Assessment Configuration** page. These parameters will be automatically applied to the protein list.

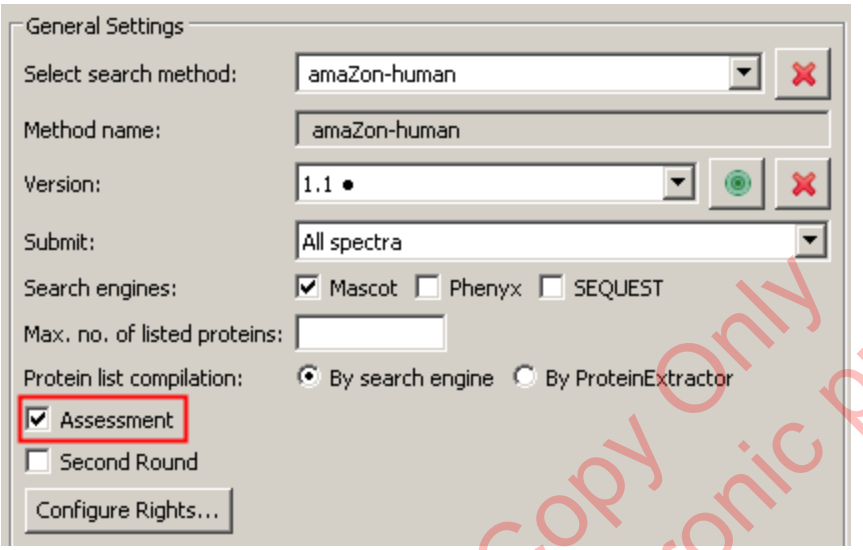

Figure 5-19 Selecting the Assessment feature in the General Settings area of the Protein Searches Parameter Configuration dialog

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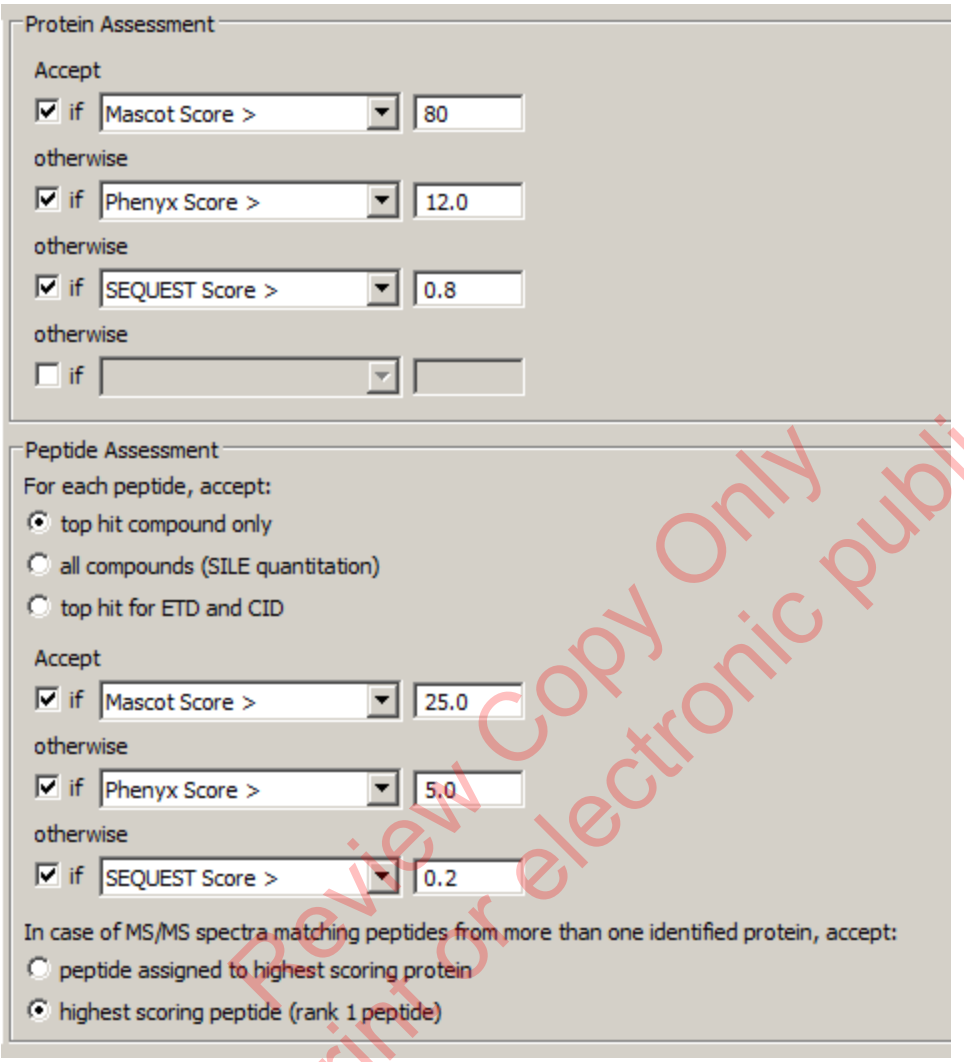

Figure 5-20 Protein and Peptide Assessment areas of the Protein Searches Assessment Configuration dialog

For protein assessment, thresholds can be set for:

- a minimum score from each search engine
- a maximum False Positive Rate. This parameter can be applied only to the ProteinScape Decoy workflow (see section 5.4.1).

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For peptide assessment, minimum score thresholds can be set for each search engine. If an MS/MS spectrum can be matched to more than one peptide (from the proteins in the result list), only one of these matches will be checked as identified. In such ambiguous cases, the user can decide whether the algorithm should check the highest-scoring peptide or the peptide belonging to the highest-scoring protein.

5.3.6 Second-Round Searches

Selecting **Second Round** in the **General Settings** area of the **Protein Searches Assessment Configuration** page enables a second round of searches using Mascot or Phenyx.

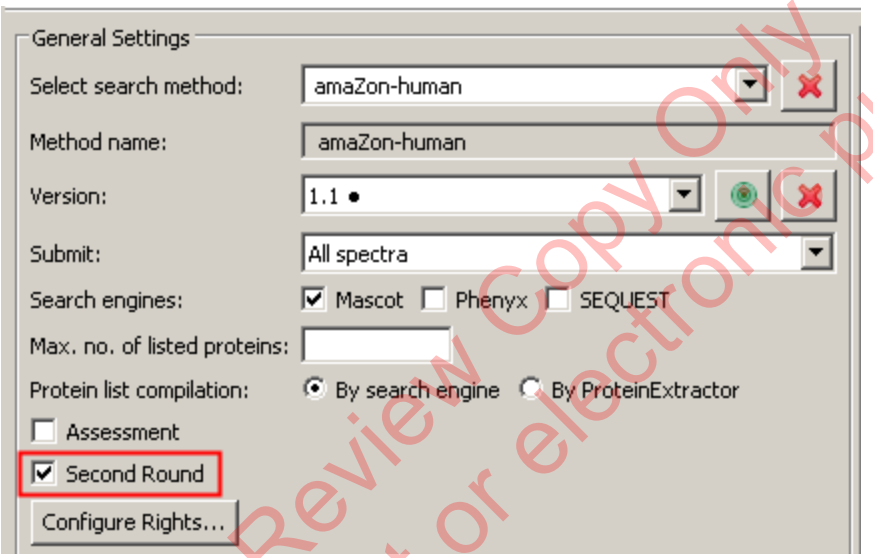

Figure 5-21 Selecting the second round of searches feature in the General Settings area of the Protein Searches Parameter Configuration dialog

The parameters used for an error-tolerant Mascot search are predefined on the Mascot server.

Parameters used for a Phenyx second round search are defined on the **Second Round Parameter Configuration** page.

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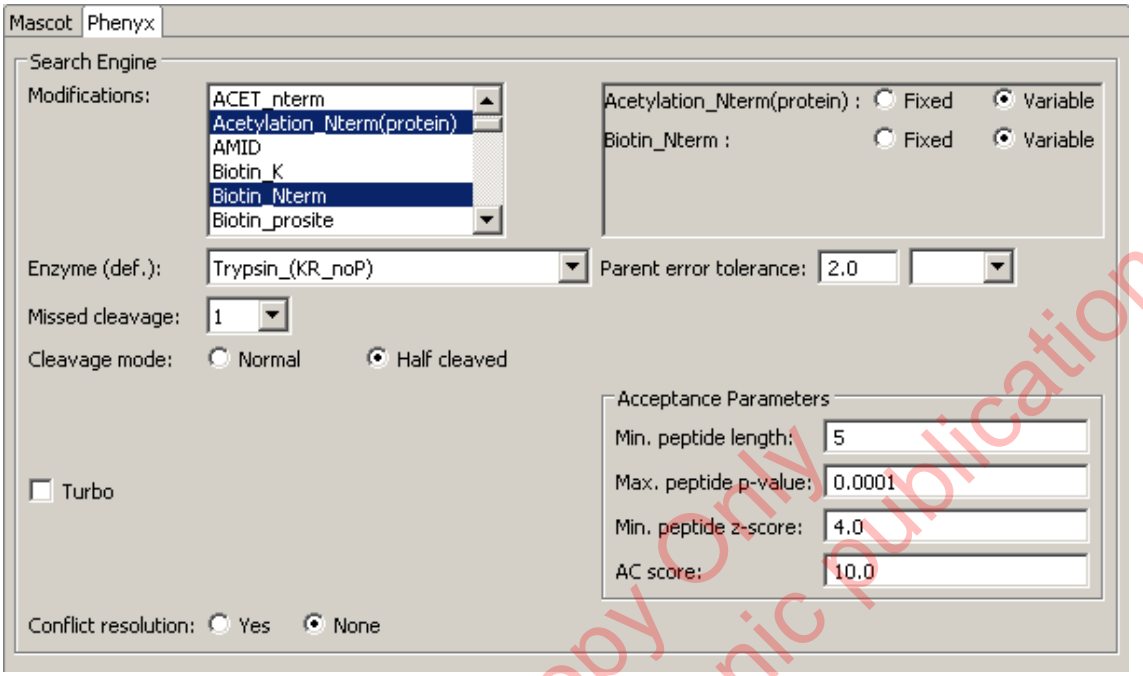

Figure 5-22 Phenyx Second Round Parameter Configuration page

See the respective search engine's documentation for further details.

5.4 Decoy Search Strategies

A combined MS/MS search generates a long list of identified proteins that are ranked using an arbitrary score. This score reflects the quality of the match between peptide and observed spectrum but does not provide a statistically meaningful measure of the result's significance.

A simple and effective tool for generating an estimation of the significance is the target-decoy search strategy. This strategy is based on the assumption that adding "decoy" sequences — which are known to be incorrect — to the searched space, will generate "incorrect" search results.

The information gained by searching this "decoy" database can be used to calculate how many incorrect results are in a set of protein identification data (the False Positive Rate, FPR). It can also be used to guide filtering parameters to increase the confidence level of correct identifications.

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5.4.1 Theory of Decoy Strategies

The decoy database is created by randomizing every sequence in the “real” database (FASTA files). This results in a second entry — which has the same mass and amino acid content but an artificial primary structure — being generated for every protein.

The decoy entries are given accession numbers that consist of the prefix “rnd” (for randomized) followed by the accession number of the original protein. The decoy database therefore consists of “real” proteins and their decoy counterparts.

A protein search against the decoy database will result in a protein list containing a number of decoy proteins, clearly identifiable by their “rnd” accession number prefix.

The decoy strategy makes the following assumptions:

- Every match to a “decoy” entry is an incorrect match (false positive)
- For every match to the “decoy” part of the database, there will also be an incorrect match to the “real” part of the database.

Therefore, the number of matches to decoy proteins is equal to the number of incorrect matches to “real” proteins. The false positive rate (FPR) is equal to:

$$FPR = \frac{Number\ of\ incorrect\ matches\ to\ "real"\ proteins}{Total\ number\ of\ "real"\ proteins\ identified}$$

In the example below, in a list of 100 proteins, two are decoy proteins. This implies that two “real” protein identifications are incorrect, giving a false positive rate of:

$$FPR = \frac{2}{98} = 2.04\%$$

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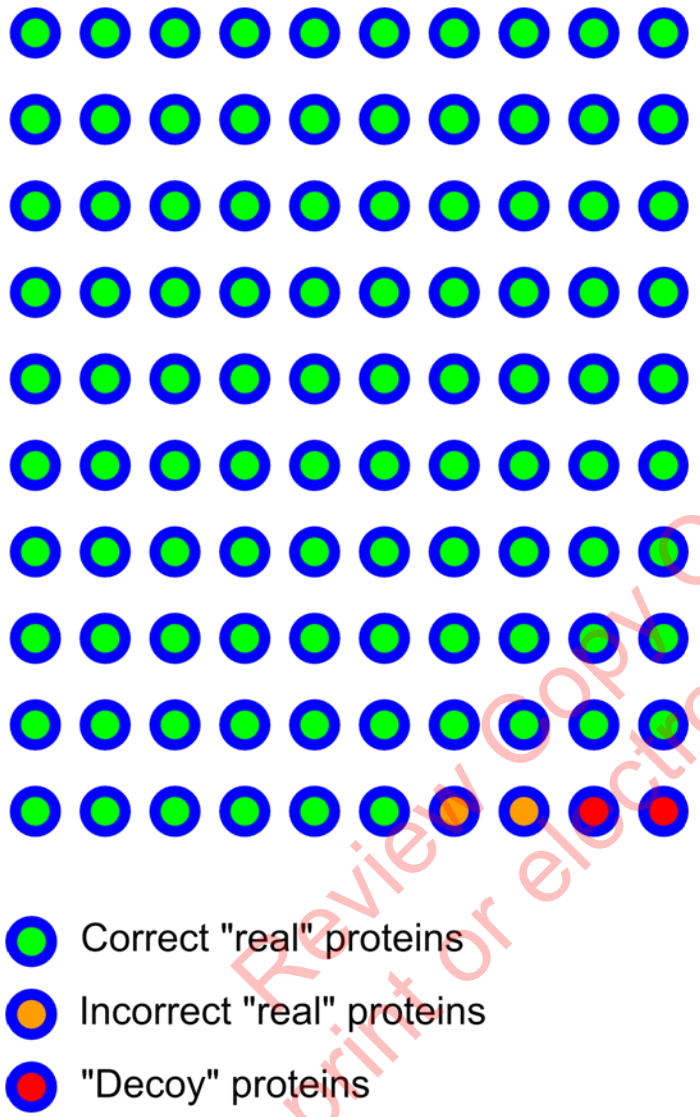

Figure 5-23 Demonstration of decoy database strategy

5.4.2 Creating and Installing Decoy Databases

Decoy databases should be created and installed by an administrator who is familiar with setting up databases on the search engine servers and in ProteinScape, and has the relevant access permissions.

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5.4.2.1 Creating a Decoy Database

Decoy databases can be created from most FASTA protein sequence database files using the Perl script `makeDecoyDB.pl`. Running this script requires a PC with a Perl interpreter.

1. Install a suitable Perl interpreter on a standard desktop PC (not the ProteinScape server!).

   ActivePerl can be downloaded from www.activestate.com/activeperl. Users must log in as Administrator before installing ActivePerl.

2. Copy the `makeDecoyDB.pl` and `makeDecoySwissprot.pl` files to a new directory on the PC.

   Note If one of the FASTA files from the ProteinScape server is used to create the decoy database, ProteinScape automatically appends the date to the file name upon indexing.

3. Copy the FASTA database file to be converted to a decoy database into the same directory and double-click `makeDecoyDB.pl`.

   A command prompt window appears asking for input.

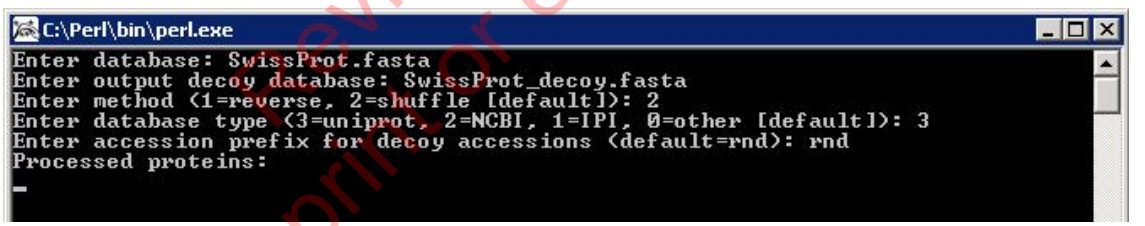
C:\Perl\bin\perl.exe
Enter database: SwissProt.fasta
Enter output decoy database: SwissProt_decoy.fasta
Enter method (1=reverse, 2=shuffle [default]): 2
Enter database type (3=uniprot, 2=NCBI, 1=IPI, 0=other [default]): 3
Enter accession prefix for decoy accessions (default=rnd): rnd
Processed proteins:

Figure 5-24 Command prompt window used to create a decoy database

- **Enter database:** Type the full file name of the FASTA file, for example, `uniprot_sprot.fasta`
- **Enter output decoy database:** Type the desired name of the decoy database including the `.fasta` suffix, for example, `uniprot_sprot_decoy.fasta`.

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- **Enter method:** Select from **reverse** (the decoy proteins are generated by reversing the original sequences) or **shuffle** (the decoy proteins are generated by shuffling the amino acids of each protein into a random order). To use the default shuffle method, press **Enter**.
- **Enter database type:** Some database formats require special handling (decoy accession numbers in IPI databases contain the decoy prefix at two positions). Specify **3** for uniprot databases, **2** for NCBI, **1** for IPI databases, or **0** for all other databases.
- **Enter accession prefix for decoy accessions:** Each decoy protein is assigned a new accession number consisting of the original accession plus a prefix (usually "rnd"). ProteinScape uses this prefix to discriminate between "real" and decoy proteins.

The decoy database is generated in the directory that contains `makeDecoyDB.pl`.

To create a decoy database from a SwissProt database, the associated `SwissProt.dat` file must also be converted. This file contains additional information about the proteins, for example, for searches with a taxonomy limitation. The `SwissProt.dat` file can be converted using the script `makeDecoySwissprot.pl`.

5.4.2.2 Installing a Decoy Database on Search Engine Servers

The decoy database must be installed on all search engine servers and also on the ProteinScape server before it can be used.

1. Copy the decoy database into the appropriate directory of the search engine server and the ProteinScape server.

   Note The relevant databases on the ProteinScape server and the search engine servers must be identical.

   Refer to the search engine's manual on how to install a new database. Usually the parsing rules can be the same as for the original database. IPI databases however, require different rules.

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2. Test the decoy database in a manual search using the web interface of the search engine.

   The decoy database must be able to be used in exactly the same way as any other active database. The search engine will not detect the decoy nature of the database. The converted *.dat file must also be installed for searches with taxonomy limitation (for example, human only) in SwissProt.

   Mascot may require adapted parsing rules. Phenyx requires databases in a precompiled form. See the Phenyx documentation for information about precompiled databases.

5.4.3 Using a Decoy Database

To use the decoy database simply select it from the drop-down **Database** list on the **Search Parameter Configuration** page.

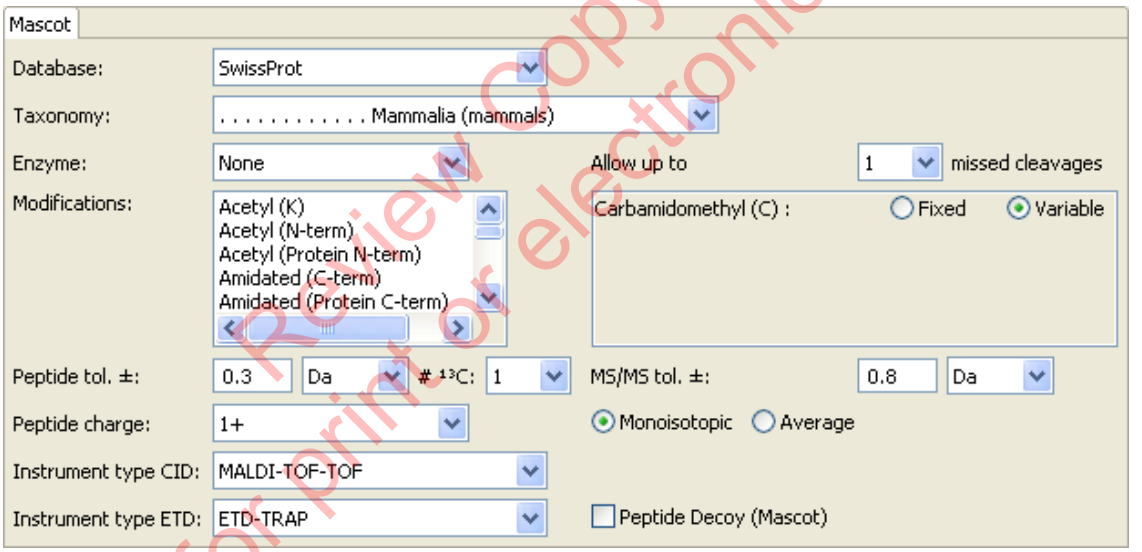

Figure 5-25 Selecting a decoy database on the Search Parameter Configuration page

Searches on any search engine can be run with or without ProteinExtractor, provided the decoy database is installed on the search engine server. The decoy strategy is currently only available for MS/MS searches.

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5.4.4 Using the Mascot Automatic Decoy Approach

Mascot offers an automatic decoy approach in which the decoy peptides are generated in real time during the protein search. Mascot calculates false positive rates (FPRs) and displays them in its web interface. Here, the user can adjust the *p*-value to reach a desired FPR and discover the respective identity score. Subsequently, a list of proteins — which contain, for example, at least one peptide that has a score above the identity threshold — can be compiled using the ProteinExtractor.

To use the Mascot decoy function, select **Peptide Decoy (Mascot)** on the **Mascot Search Parameter Configuration** page. Selecting **Peptide Decoy (Mascot)** also enables the **Percolator** feature — a post-search algorithm to recalculate peptide scores on the basis of a statistical analysis — to be applied (Mascot 2.3 or later).

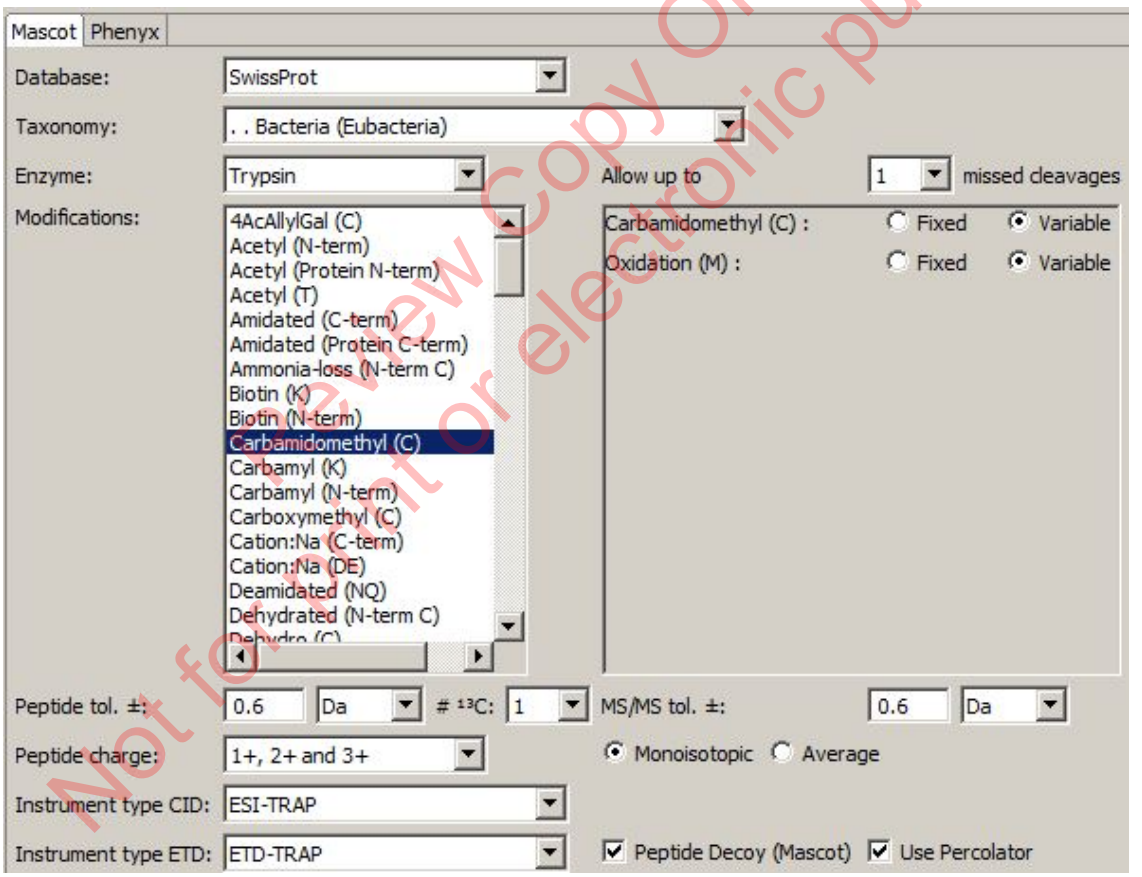

Figure 5-26 Select Peptide Decoy (Mascot) to activate the Mascot decoy function

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5.5 Processing Search Results

The protein list compilation functionality of ProteinExtractor can be applied to a single search result or to merged peptide lists from multiple searches (see section 5.5.1). Protein list can be compiled from searches that were performed using:

- different parameters (instrument settings, mass tolerances, and so on);
- different data sets;
- different instruments (LC-MALDI + LC-ESI, CID + ETD, and so on);
- more than one search engine.

In addition, new assessment parameters can be defined for existing search results to increase or decrease the stringency of the protein acceptance criteria (see section 5.5.2).

5.5.1 Compiling Protein Lists from Existing Protein Searches

►► To generate a new protein list from one or more existing search results

1. Select the desired search results:
   a. Select one or more pink Search Result node(s) . To select multiple search results, press **CTRL** while selecting nodes.
   b. Right-click the selection(s) and select **Search Result Processing > Protein List Compilation** from the menu.
   c. Select a destination in the **Project Navigator** tree and enter a **Search result name**.
      - Alternatively, right-click the node containing the desired search results and select **Search Result Processing > Protein List Compilation** from the menu. Select the desired search results in the Protein List Compilation dialog and click **Next** Select a destination in the **Project Navigator** tree and enter a **Search result name**.

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2. Click **Next >**.
3. The **Protein List Compilation Search Parameter Configuration** dialog box opens. Note that the search engine parameters are unavailable and cannot be changed. The **General Settings**, **ProteinExtractor** settings, and **Protein** and **Peptide Assessment** settings can be changed.
4. Use the **< Back** and **Next >** buttons at the bottom right of the dialog box to navigate between the dialog box pages. Click **Start** to begin the database search and **Cancel** to exit the dialog box.

5.5.2 Defining New Assessment Parameters for Existing Results

►► To define new Protein Assessment parameters for one or more existing search results

1. Select and right-click **Search Result** node(s) and select **Search Result Processing > Assessment** from the shortcut menu.

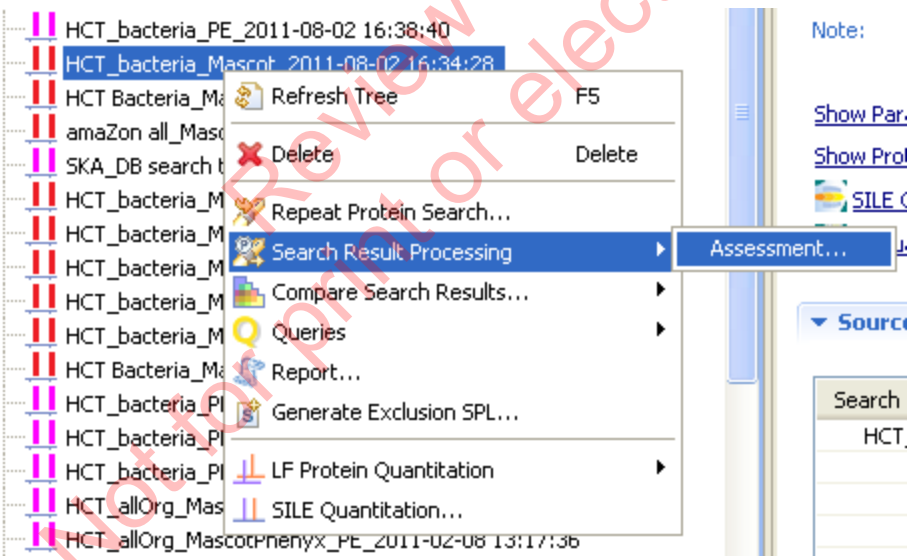

Figure 5-27 Defining new assessment parameters

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2. The **Protein List Compilation Search Parameter Configuration** dialog box opens.

   Note that the search engine parameters and **ProteinExtractor Settings** are unavailable and cannot be changed. The **General Settings** and **Protein-** and **Peptide Assessment** settings can be changed.

3. Use the **< Back** and **Next >** buttons in the lower-right corner of the dialog box to navigate between the dialog box pages. Click **Start** to begin the database search and **Cancel** to exit the dialog box.

5.6 Performing Peptide Mass Fingerprint (PMF) Searches

Peptide Mass Fingerprint (PMF) searches are launched from single MS spectra data sets (). For PMF searches, the protein list is directly reported by the search engine. For this reason, the **Protein List Compilation** page of the **Protein Searches** dialog is not available when configuring PMF searches.

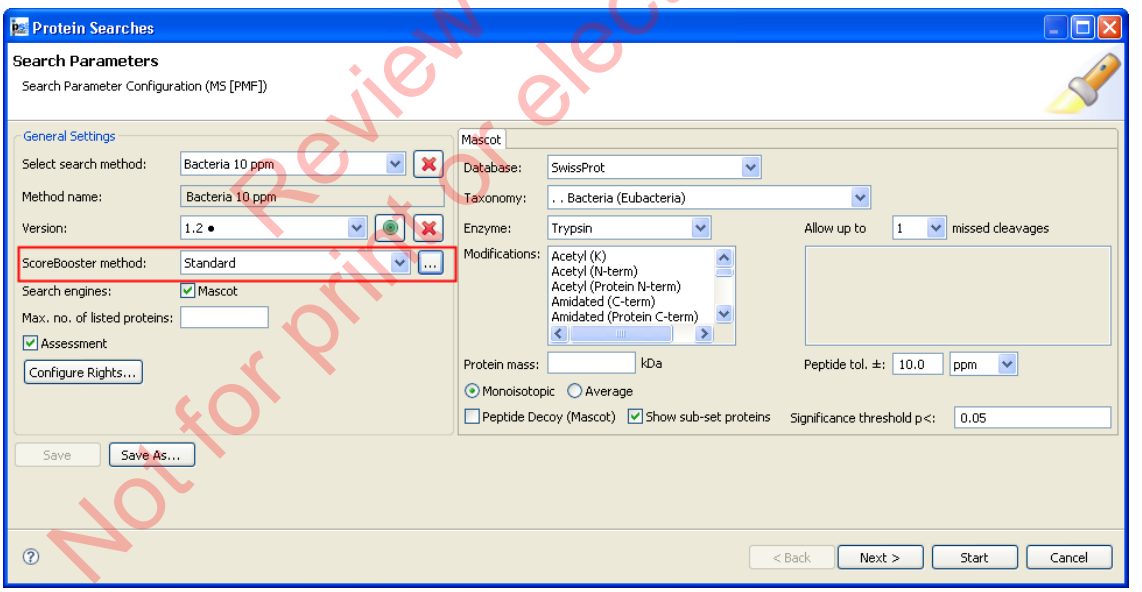

Figure 5-28 PMF Protein Searches dialog Search Parameters page

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The search parameters used for PMF searches are essentially the same as those used for peptide identification by MS/MS (see section 5.3.1), with the obvious exception of MS/MS tolerance, charge limitations, and so on.

5.6.1 Selecting the Data used for Protein MS Searches

Protein MS searches can be launched from nodes containing data sets or the data set nodes themselves. Launching a search from a non-data node (Project, Sample, LC, Gel, or Digest) enables the user to select and filter the data sets used for the search.

►► To select and filter data used for protein searches (launch search from a non-data node)

1. Right-click a non-data node (Project, Sample, LC, Gel, or Digest) containing the desired data set(s) and select **Protein Search** from the shortcut menu.

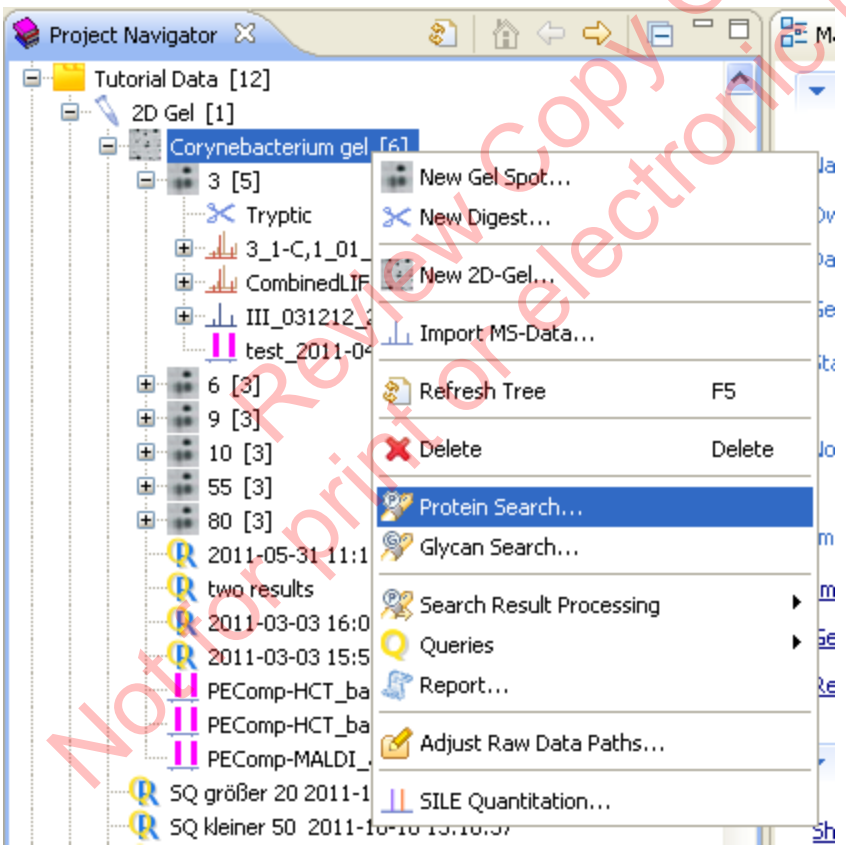

Figure 5-29 Launching a protein search from a 2D Gel node in the Project Navigator

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The **Protein Searches > Spectra Selection** dialog box opens.

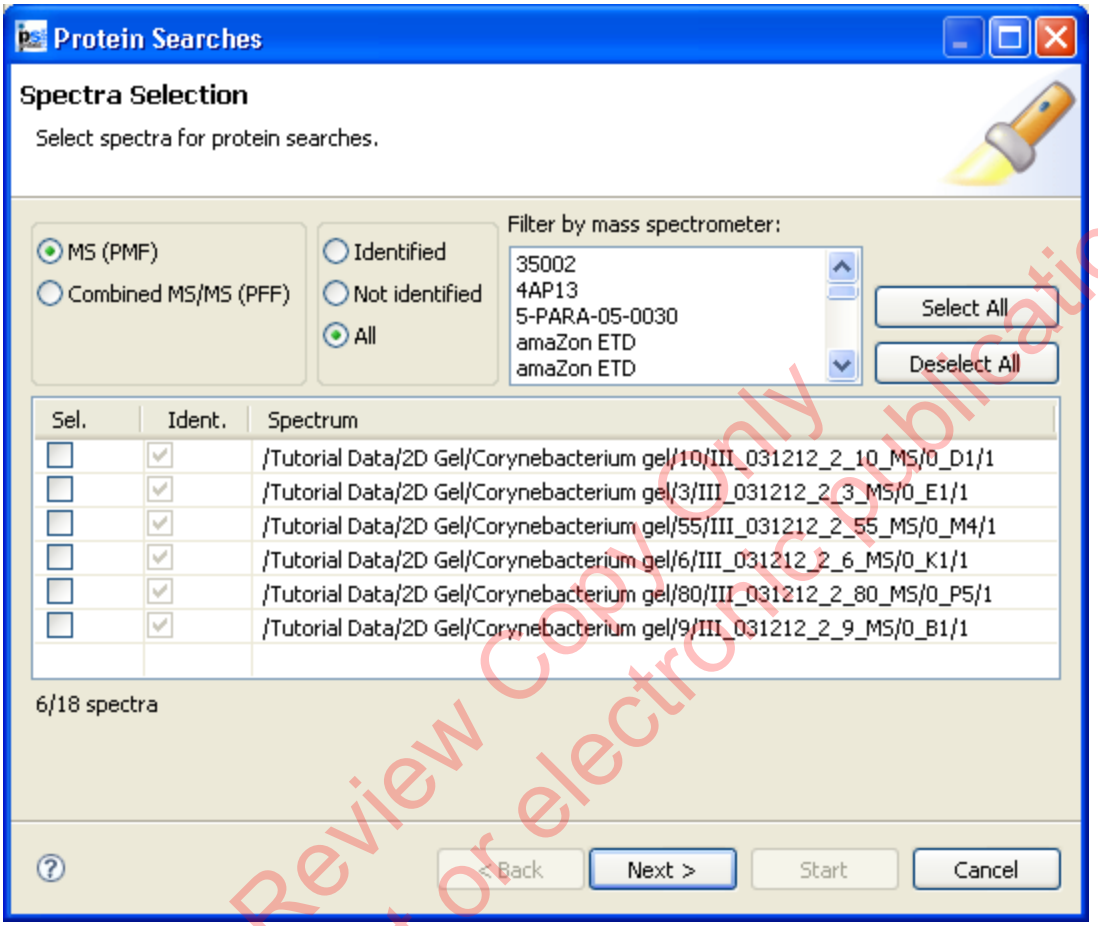

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Figure 5-30 Protein Searches > Spectra Selection dialog

2. Select the data type **MS (PMF)** and identification status (**Identified**, **Not identified** or **All**).

   Selecting **MS (PMF)** will list all MS data sets in the node.

   The list of data sets can be filtered by selecting one or more mass spectrometers from the **Filter by mass spectrometer** list.

3. In the **Sel.** column, select the box next to the data that will be used for the protein search.

Use **Select All** or **Deselect All** to include or remove all data sets from the selection.

4. Click **Next**.

   The **Protein Searches** dialog opens (see section 5.3).

►► To launch a protein search directly from an MS data node

- Right-click a Combined Data ( ) or MS Data ( ) node and select **Protein Search** from the shortcut menu.

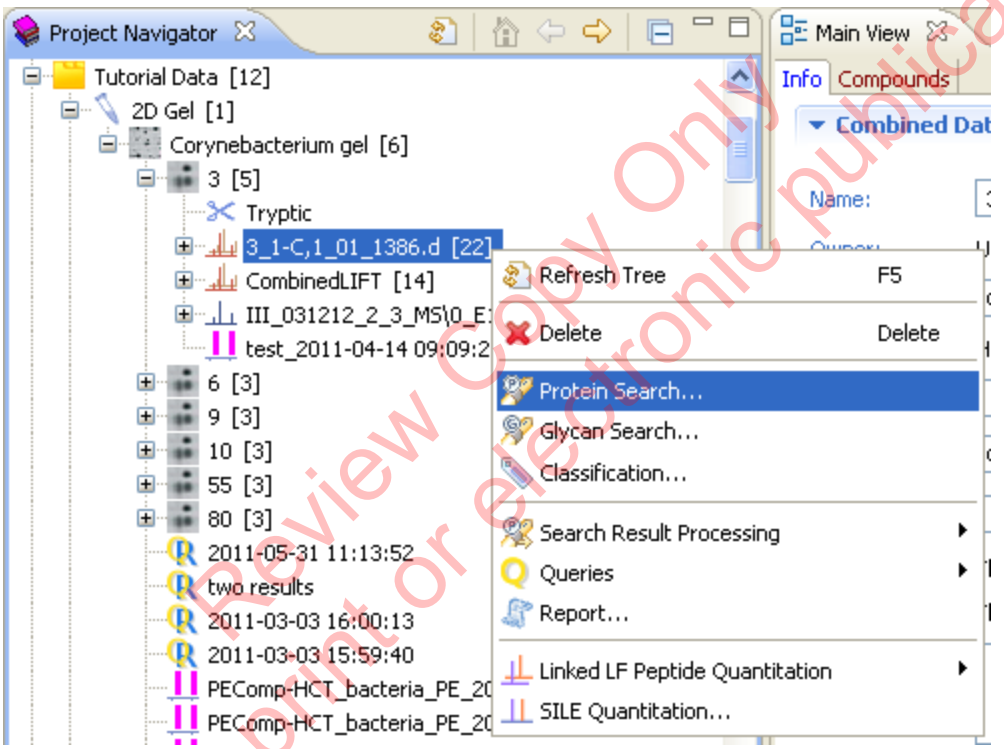

Figure 5-31 Launching a protein search from a Combined Data node in the Project Navigator

The **Protein Searches** dialog opens (see section 5.3). The type of data (MS [PMF]) is indicated in parentheses in the dialog header.

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5.6.2 ScoreBooster

A feature that is currently only available for MS searches is ScoreBooster.

ScoreBooster is an algorithm based on a Calibrant list (Mass Control List, MCL) of known background and calibrant masses. It labels known peaks as background (contaminants, polymer distributions, and so on) so they are not submitted to searches. It also performs an internal calibration using either the masses of the Mass Control List or the peptides of an identified protein as calibrants. The ScoreBooster is controlled by a ScoreBooster method which can be specified in the MS search method (see 5.6.2). A calibrant list and further parameters can be defined in the ScoreBooster Method dialog (see Figure 5-32).

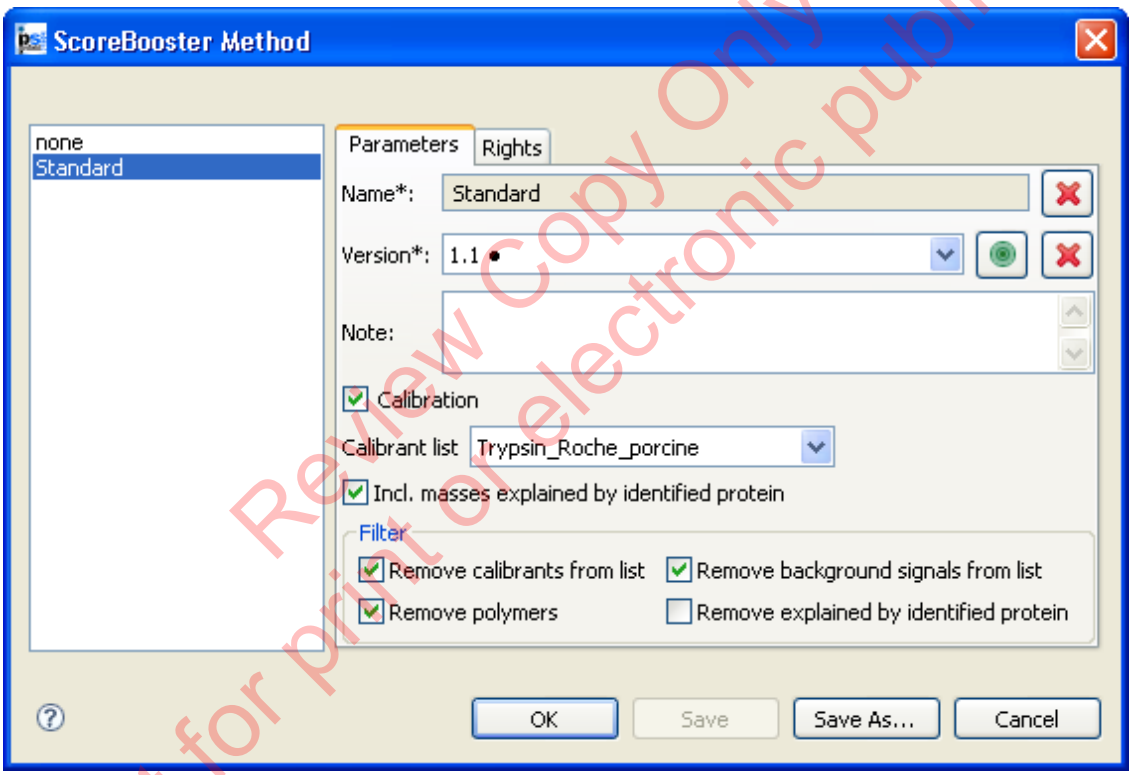

Figure 5-32 ScoreBooster Method dialog

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Table 5-4 ScoreBooster parameters

Parameter	Description
Calibration	Use the masses of the defined calibrant list for an internal calculation of the spectrum before the search.
Incl. masses explained by identified protein	Use peaks from identified proteins as additional calibrant peaks.
Remove calibrants from list	Calibrant peaks will not be submitted to the search.
Remove background signals from list	Calibrant list masses flagged as background will not be submitted to the search.
Remove polymers	Regularly distributed signals recognized as polymers will not be submitted to the search.
Remove explained by identified protein	Peaks from identified proteins will not be submitted to subsequent searches.

After a search using the ScoreBooster, a new peak list is generated for the spectrum. By default, the Spectrum Viewer shows the most up-to-date peak list. Right-click the Spectrum View and select **Select spectrum** to view previously obtained peak lists.

5.6.3 Protein Assessment

The user can decide which of the proteins to accept. This can be done either interactively, or assessment parameters can be defined that are then applied automatically (see section 5.3.5). Peptide Assessment cannot be applied to PMF searches.

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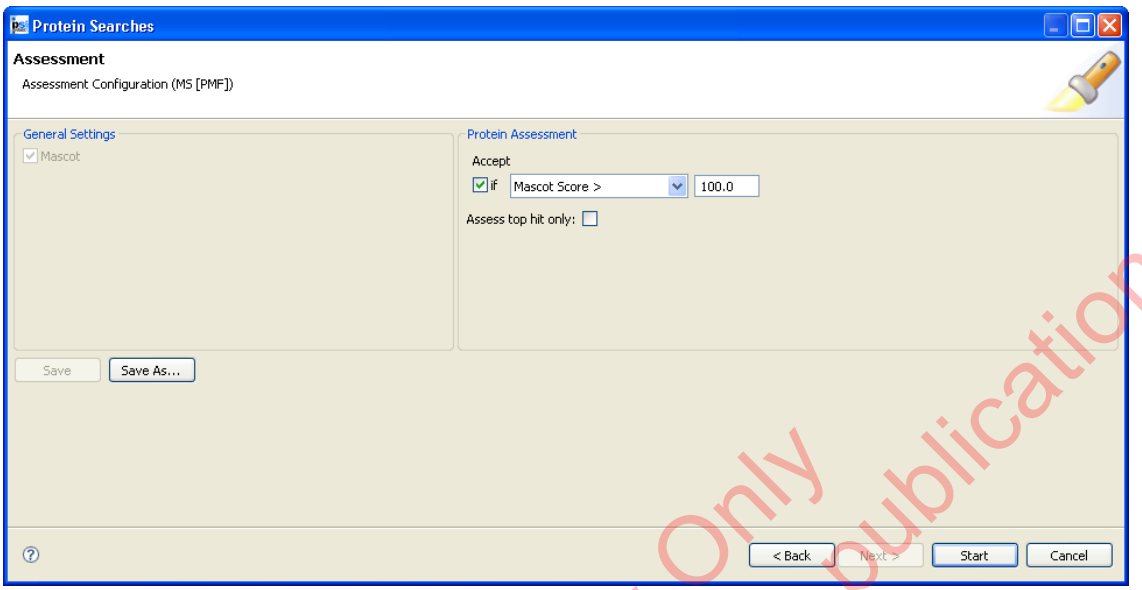

Figure 5-33 PMF Protein Searches dialog Assessment page

5.7 Starting and Repeating Protein Searches

A protein search is started by clicking **Start** on any of the **Protein Searches Parameter Configuration** pages.

To repeat a protein search, select **Repeat Protein Search** in the shortcut menu of an existing **Search Result** node, or **Repeat Search** in the **Info > Search Result Info** area of its Main View.

Selecting either of the above options for repeating a search opens the **Protein Searches Parameter Configuration** dialog box where any search parameter can be changed. Click **Start** to begin the new search.

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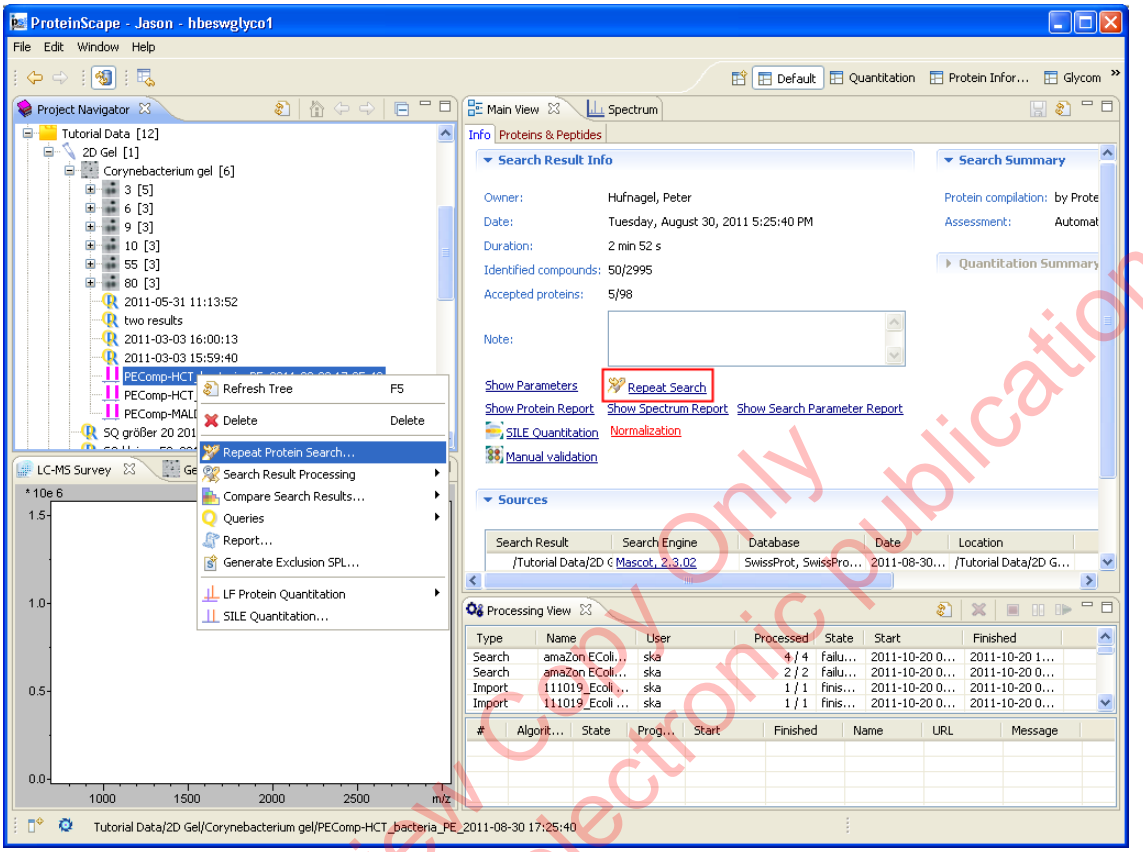

Figure 5-34 **Repeating a protein search using (left) the shortcut menu or (right) Repeat Search in the Main View**

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5.8 Configuring Automatic Searches

The **Automatic MS** and **Automatic MS/MS Searches** areas of the **Sample** Main View enable new methods to be automatically applied to a new peak list assigned to the selected **Sample** node.

If an automatic search is configured and a data set from a selected **Spectrometer model** is transferred to the sample or any sub-node of the sample, the specified search method(s) will be automatically triggered.

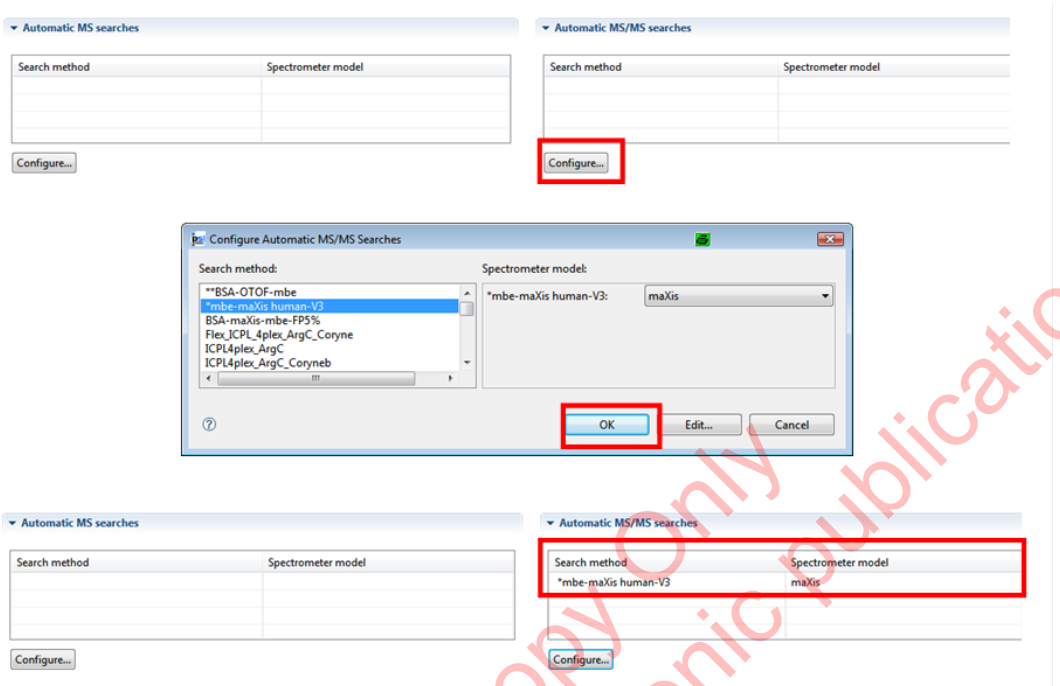

Figure 5-35 Configure Automatic MS/MS Searches dialog

►► To configure automatic searches

1. Click **Configure** in the **Automatic MS** or **Automatic MS/MS Searches** area of a **Sample** Main View.
2. Select one or more **Search method**s and select a **Spectrometer model** from the drop-down list for each selection.
3. Select individual methods and click **Edit** to change search parameters.
4. Click **OK** to close the dialog box.

   The **Search method**(s) and **Spectrometer model**(s) are listed in the Automatic MS / MS/MS Searches areas of the Sample Main View.

5.9 Following the Progress of Searches

The progress of the database search can be followed in the **Processing View**, which is opened by selecting **Window > Show View > Processing View** (see section 6.4.6).

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5.10 Creating Feedback Loops for MS/MS Acquisition with Flex Series Instruments

ProteinScape offers feedback loops for intelligent MS/MS acquisition from MALDI single spectra. The following steps can be automated in a single workflow:

- Acquire an MS spectrum
- Trigger a PMF search on the Mascot server
- Retrieve and assess the result
- Based on the identification status, acquire a number of MS/MS spectra of identified peaks (for validation) and unidentified peaks (for example, for identification of background signals, modified peptides an so on.)

To perform an automatic acquisition and analysis:

- An AutoXecute run must be set up that assigns the acquired spectra to a number of gel spots.
- Before starting the acquisition, a method for automatic protein searching (see section 5.8) must be specified at the **Sample** level. This method will be triggered whenever a spectrum is transferred to any node below this **Sample**.
- Automatic Assessment must be enabled in the PMF (MS) Search Method, (see section 5.3.5).
- A WARP (Workflow Administration by Result-driven Processing) method must be specified in the WARP (automatic MALDI MS/MS acquisition) section in the Main View of the relevant **Sample**. Click the **Browse** button to the right of the **WARP method** drop-down list to open the WARP method dialog (Figure 5-36 and Table 5-5).

Note This workflow was developed for the analysis of 2D Gels. It can be used for the analysis of other sample types, but the Project Navigator containers for the spectra must be a 2D Gel and Gel Spots.

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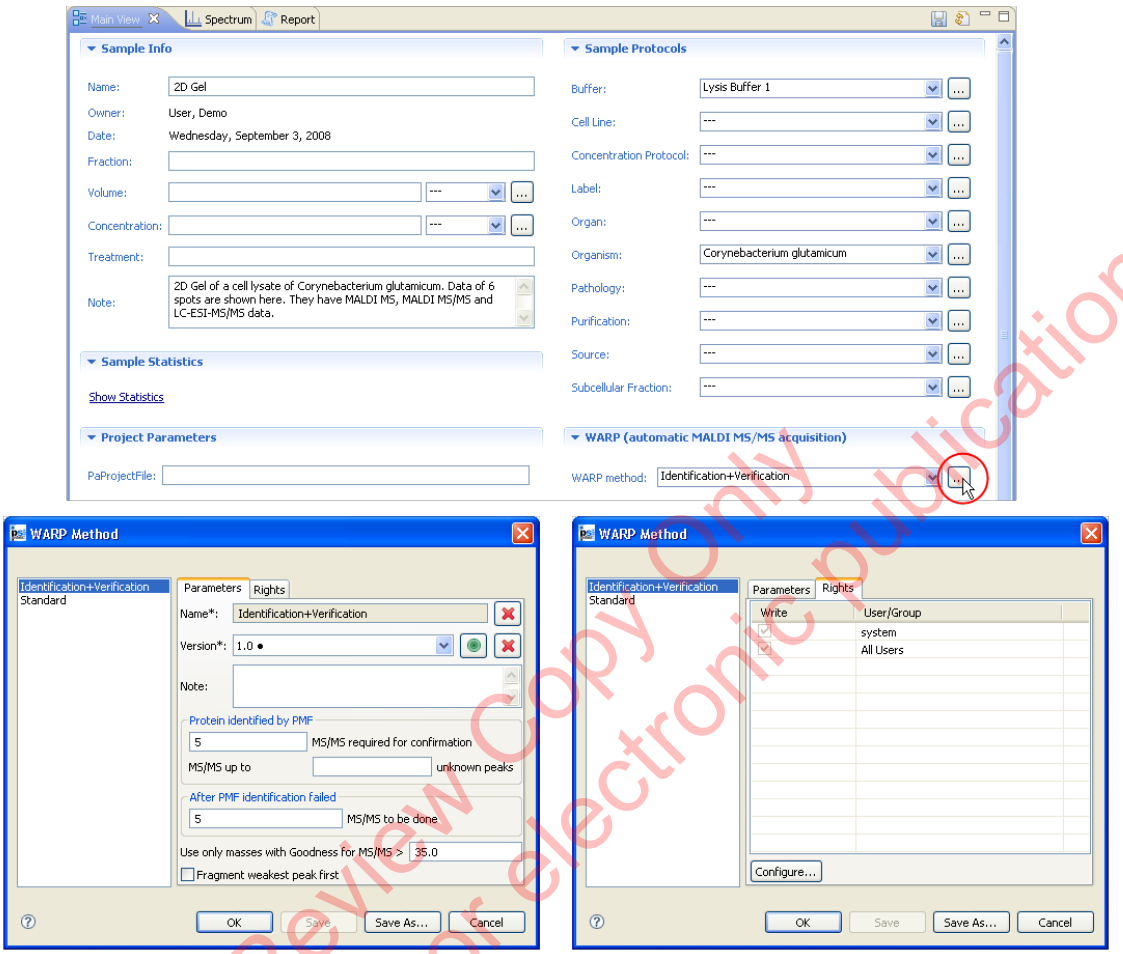

Figure 5-36 **WARP (automatic MALDI MS/MS acquisition) section in the Main View (top) and WARP Method dialog Parameters page (lower left) and Rights page (lower right)**

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Table 5-5 WARP Method dialog parameters

Parameter	Description
Protein identified by PMF	
…MS/MS required for confirmation	For those target positions with identified proteins (at least one OK check-mark in a Search Result under the MS spectrum) use the given number of identified peaks for validation of the identified protein.
MS/MS up to … unknown peaks	For those target positions with identified proteins, select the given number of peaks that do not match the identified protein. This enables closer investigation of background peaks, for instance to discover whether a second protein or unexpected cleavages and modifications are present.
After PMF identification failed	
… MS/MS to be done	For those target positions that did not yield an identification, acquire the given number of MS/MS spectra. Using this feature, there is a chance to obtain some good MS/MS spectra that may lead to an identification, even if the PMF search failed.
Use only masses with Goodness for MS/MS >…	A**Goodness for MS/MS** score is calculated for every peak. The higher the intensity, the larger the score. Additional peaks in the vicinity and a high mass will decrease a peak's score. A mass of < 900 will strongly decrease the score. Usually, a threshold value for this parameter is 180. The **Goodness for MS/MS** of each peak is displayed in the peaklist table (see Figure 5-37).
Fragment weakest peaks first	MS/MS spectra are usually acquired in an order of decreasing "Goodness for MS/MS". Selecting **Fragment weakest peaks first** reverses the order of acquisition. This means that peaks that are likely to produce good MS/MS spectra even if little sample is available are measured later than weaker peaks that may require more sample to produce good spectra. For most instruments / preparations this option should be cleared.

Usually, this workflow is executed in an automated fashion. However, it can be influenced by manual interaction. After MS acquisition, a number of searches can be started manually and the protein identification can be refined. In this case, the WARP calculation must be manually triggered before MS/MS acquisition. This is performed in the Main View of the MS spectrum (see Figure 5-38). Additionally the peaks used for MS/MS can be manually selected in the **Do MS/MS** column of the peak list (see Figure 5-37).

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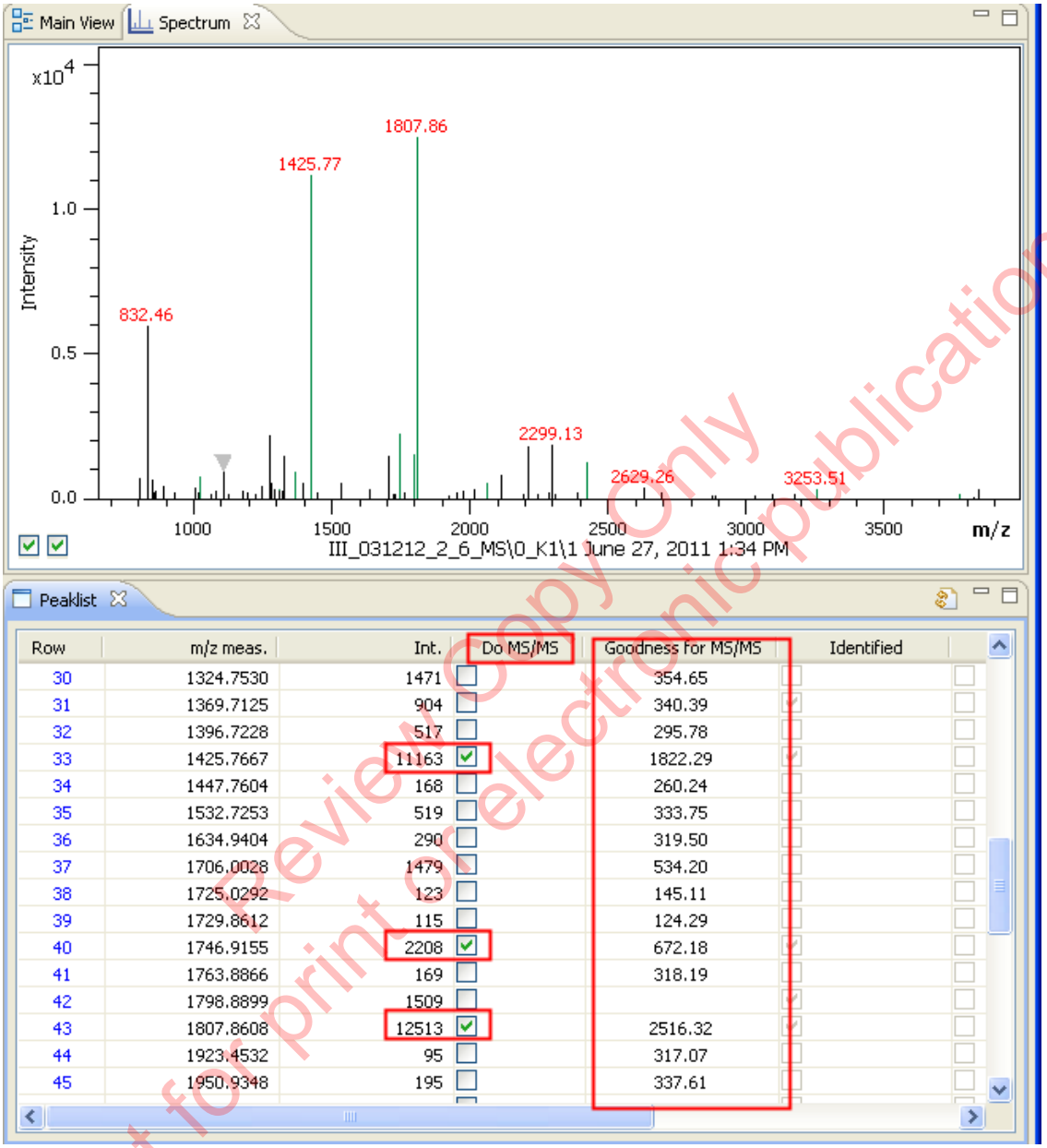

Row	m/z meas.	Int.	Do MS/MS	Goodness for MS/MS	Identified
30	1324.7530	1471	☐	354.65	☐
31	1369.7125	904	☐	340.39	☐
32	1396.7228	517	☐	295.78	☐
33	1425.7667	11163	☑	1822.29	☐
34	1447.7604	168	☐	260.24	☐
35	1532.7253	519	☐	333.75	☐
36	1634.9404	290	☐	319.50	☐
37	1706.0028	1479	☐	534.20	☐
38	1725.0292	123	☐	145.11	☐
39	1729.8612	115	☐	124.29	☐
40	1746.9155	2208	☑	672.18	☐
41	1763.8866	169	☐	318.19	☐
42	1798.8899	1509	☐		☐
43	1807.8608	12513	☑	2516.32	☐
44	1923.4532	95	☐	317.07	☐
45	1950.9348	195	☐	337.61	☐

Figure 5-37 PMF spectrum and peak list

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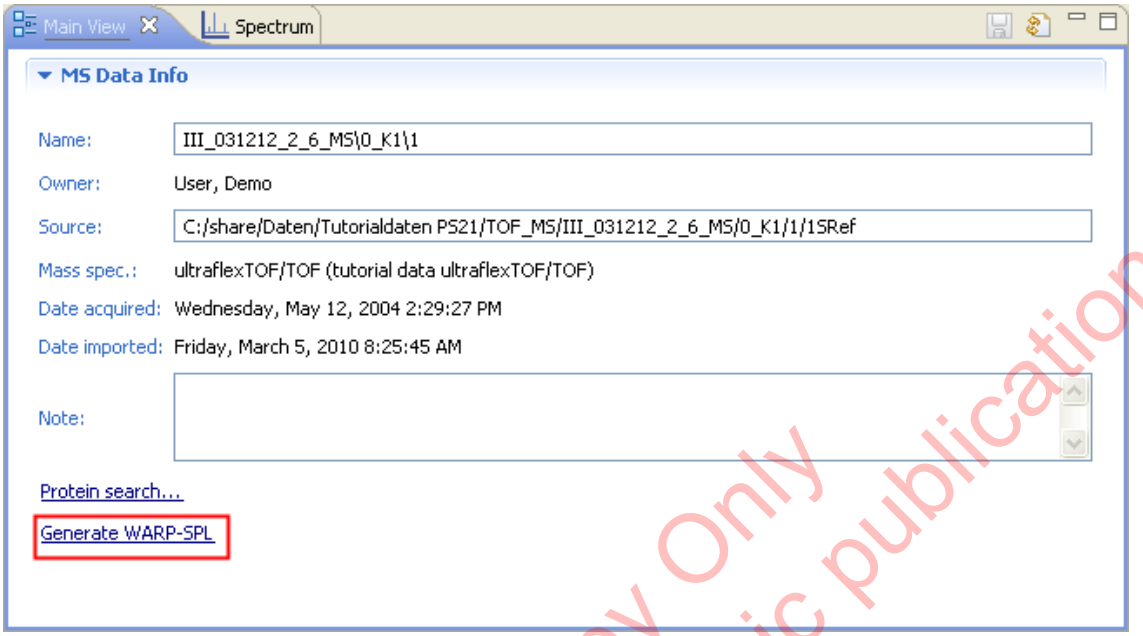

Figure 5-38 Generate WARP-SPL function in PMF spectrum Main View

Note Two important parameters must be set in AutoXecute: **Feedback from ProteinScape** must be selected in the **AutoXecute Settings** page and **WARP** must be enabled in the **AutoXecute Method Editor** (see Figure 5-39).

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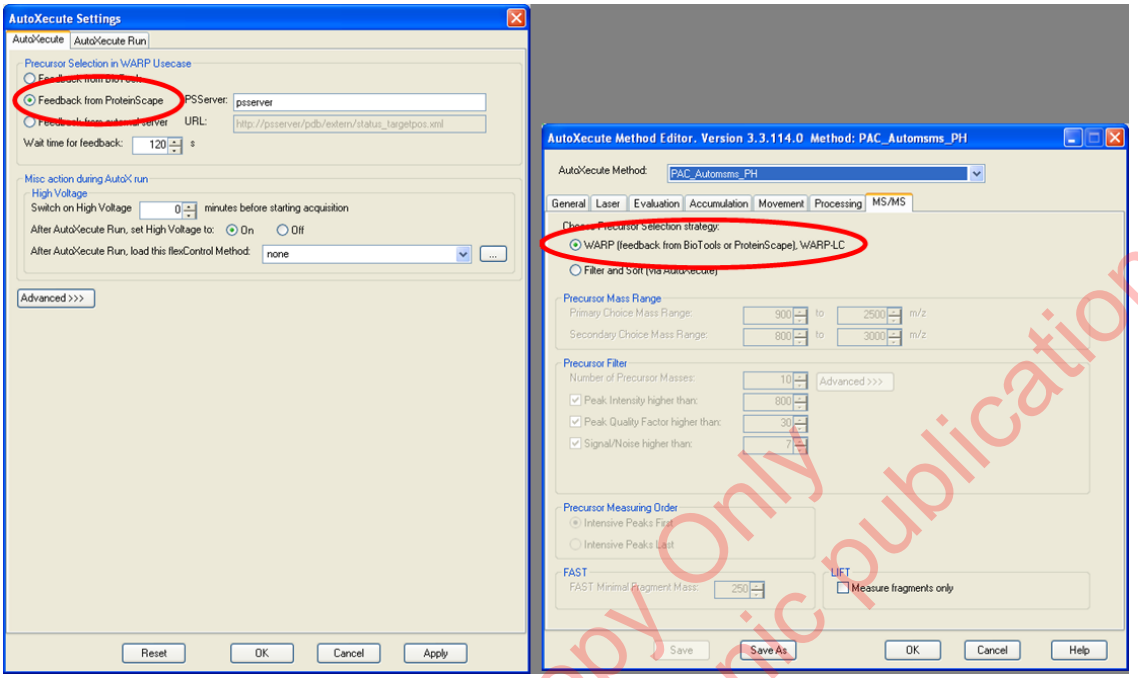

Figure 5-39 Parameters that must be set in AutoXecute to enable automatic analysis

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6 Evaluating and Displaying Protein Search Results

6.1 Protein Search Result Main View

The Main View of a protein **Search Result** node consists of two pages:

- Info page
- Proteins & Peptides page

Clicking a tab in the upper-left corner of the Main View moves the respective page into the foreground (see Figure 6-1).

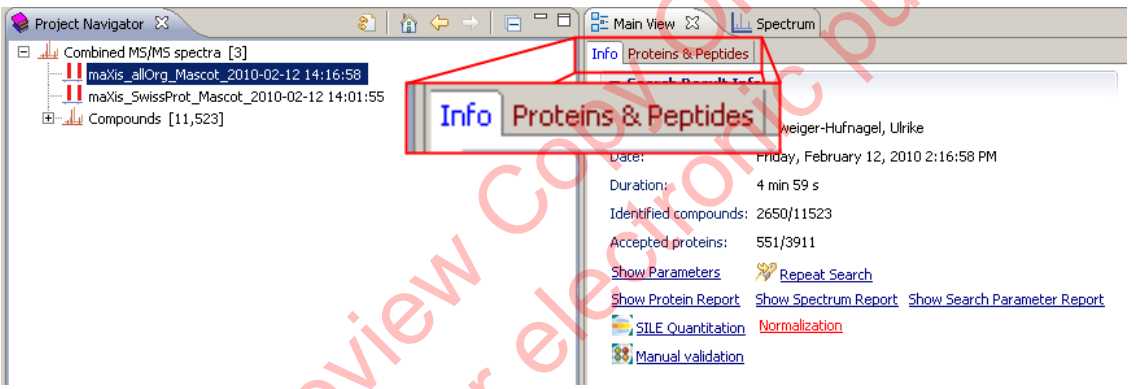

Figure 6-1 **Info and Proteins & Peptides page tabs in a Protein Search node Main View**

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6.1.1 Protein Search Info Page

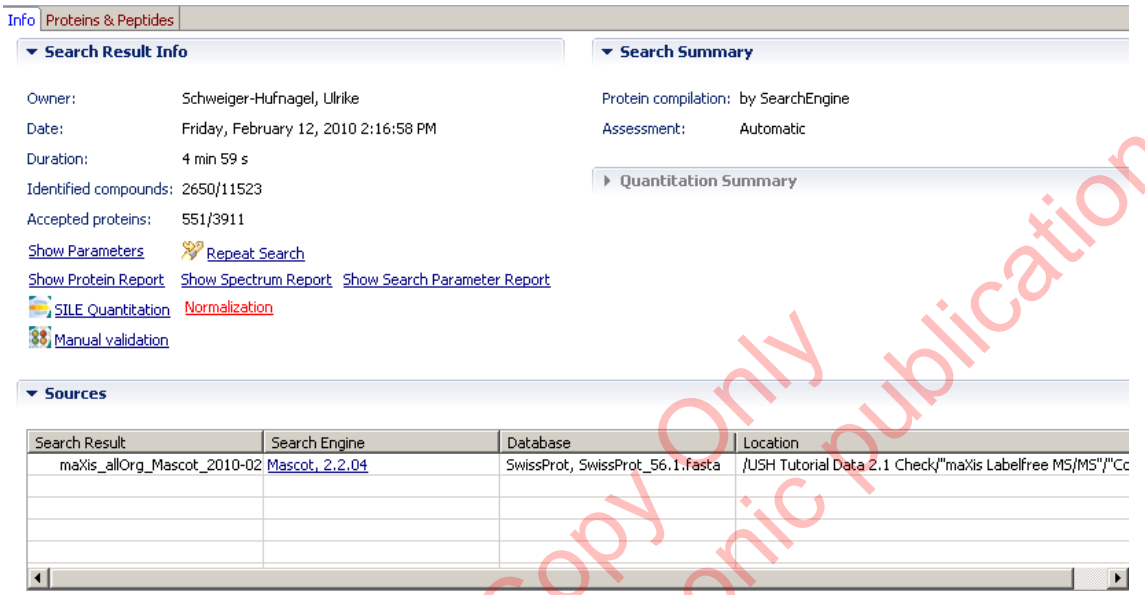

Figure 6-2 Info page of the protein Search Result Main View

The **Search Result Info** area of the Search Result Main View **Info** page displays:

- **Owner**: — the project owner
- **Date**: — date and time that the search was started
- **Duration**: — time required for the search
- **Identified compounds**: — the number of identified compounds
- **Accepted proteins**: — the number of accepted proteins

It also contains buttons for additional actions.

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Table 6-1 Additional action buttons on the protein search Info page

Action	Description
Show Parameters	Displays the **Search Parameter** dialog box pages with the parameters used for the search. Parameters cannot be altered.
Repeat Search	This button is active if the **Search Method** applied has been saved. Clicking this button opens the **Search Parameter** dialog box, where parameters can be changed and a new search performed.
Show Protein Report	Displays a detailed report of all accepted proteins (see section 10.1).
Show Spectrum Report	Displays the spectrum information and spectrum for each protein that was identified by one peptide only (see section 10.4).
Show Search Parameter Report	Displays a summary of the search parameters (see section 10.5).
SILE Quantitation	Perform quantitation in Stable Isotopic Label Experiments (SILE).
Normalization	Set parameters for normalizing quantitation ratios for SILE quantitation.
Manual Validation	A whole search result, individual peptides or individual unidentified compounds can be sent to BioTools for manual validation purposes. Here, various features such as **Search for Masses** are available. The revised proteins and peptides can be sent back to ProteinScape as a new Search Result (see section 6.4.3). Result (see section 6.4.3).

Note Additional actions that are not available (for example, **Repeat Search** for an unsaved search method) are colored red.

The **Search Summary** area shows the applied protein compilation algorithm (**by SearchEngine** or **by ProteinExtractor** and the type of assessment.

The **Quantitation Summary** area displays the **Quantitation method**, **Quantitation status**, and **Manual interaction**.

The **Sources** table displays an overview of the search results within a node, their location, the date they were performed, and the search engine and database used. This overview is extremely useful when multiple search results have been compiled in a single node.

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Right-click a search result in a list and select **Navigate to Search Result** to go directly to the corresponding result in the **Project Navigator** tree.

Table 6-2 Sources table

Column Name	Description
Search Result	Search result file name appended with start date and time.
Search Engine	Hyperlink to search results displaying search engine and version number used (for example, Mascot, 2.2.04).
Database	Database used for search.
Date	Date and time that search was started.
Location	Location of search result file.

6.1.2 Proteins & Peptides Page

The **Proteins & Peptides** page of the **Search Result** Main View displays information on the identified proteins and peptides in tabular form.

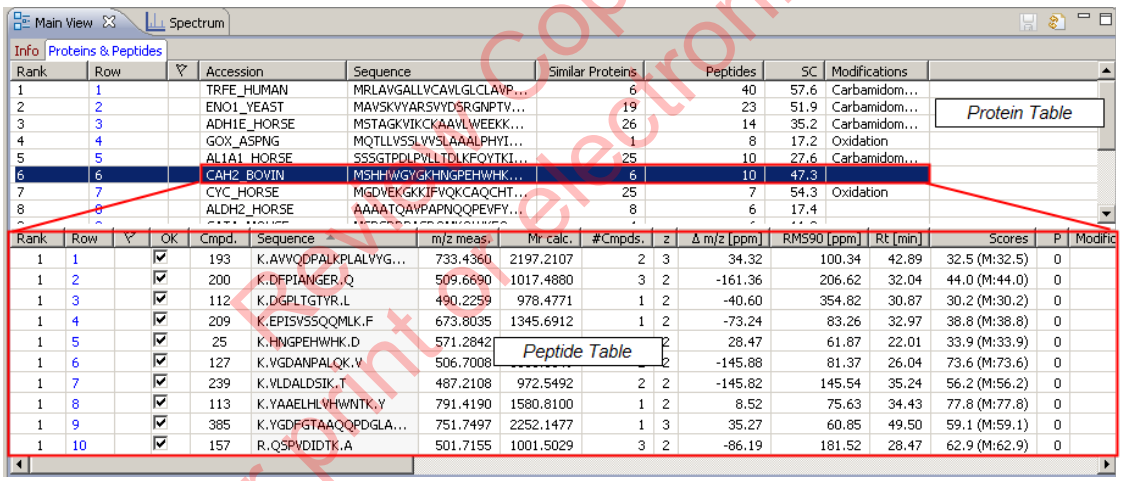

Figure 6-3 Proteins & Peptides page of the protein Search Result Main View

The upper part of the page displays a list of accepted proteins ranked by number of identified peptides; the lower part displays the list of peptides identified in the selected protein.

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6.1.2.1 Protein Table

The protein table displays a wide range of information about the proteins accepted as having been identified in the search.

Column	Description
Rank	Ranking according to number of identified peptides.
Row	Row counter.
Flag	Assign a colored flag by right-clicking a protein entry and selecting **Flags** from the shortcut menu.
OK	Selected box indicates protein is accepted as identified. The accepted status is automatically assigned during the search. Status can be changed manually by clearing box or using the shortcut menu.
Accession	Database accession name
Protein	Protein name.
Sequence	Protein sequence.
DB Name	Name of database.
MW [kDa]	Molecular weight of protein.
pI	Isoelectric point of protein.
Similar proteins	Number of similar proteins (subset matches) that can be found in the Alternative Proteins View.
Scores	Protein score in the format score (M: Mascot score; P: Phenyx score; S: Sequest score).
Peptides	Number of peptides identified.
SC	Sequence coverage (%).
Modifications	Chemical modifications present.
RMS / RMS90 [Da] / [ppm]	Deviation from predicted mass (root mean square value / root mean square 90% confidence value).
Rank	Ranking based on number of identified peptides.

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6.1.2.2 Peptide Table

The peptide table displays information about the accepted peptides identified in the protein selected in the protein table.

Column	Description
Row	Row index
Flag	Assign a colored flag by right-clicking a protein entry and selecting **Flags** from the menu.
OK	Selected box indicates peptide is accepted as identified. The accepted status is automatically assigned during the search. Status can be changed manually by clearing box or using the shortcut menu.
Cmpd.	Compound number.
#Cmpds.	Number of compounds found in the LC-MS/MS data sets for a given peptide.
***m/z* meas. / calc.**	Measured /calculated mass-to-charge ratio.
MH+ meas. / calc.	Measured /calculated MH+ value.
Mr meas. / calc.	Measured /calculated Mr value.
z	Charge.
Accession	Database accession name.
Protein	Protein name.
Sequence	Peptide sequence.
Rt (min)	Retention time in minutes.
P	Partials (missed cleavages).
Type	Fragmentation type.
Range	Position in protein (aa number to aa number).
Scores	Protein score in the format score (M:score).

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Column	Description
Δ *m/z* [Da] / [ppm]	Difference in mass-to-charge ratio.
Δ MH+ [Da] / [ppm]	Monoisotopic mass difference.
Modifications	Chemical modifications present.
RMS / RMS90 [Da] / [ppm]	Deviation from predicted mass (root mean square value / root mean square 90% confidence value).
Rank	Ranking based on number of identified peptides.
Int.	Precursor ion intensity.
AltPep.	Number of alternative matches.
IntCov. [%]	Intensity coverage (%).
Location	Location of source file.
Dataset	Dataset file name.
Search Result	Search result file name.
Fraction	Fraction.

6.1.2.3 Proteins & Peptides Table Shortcut Menu

The information displayed in the protein or peptide table can be configured using the respective shortcut menu, which is launched by right-clicking anywhere in the table. The shortcut menu contains the common table view commands (see section 2.6). and the following additional commands.

Show — Protein-specific (see section 6.2) or peptide-specific (see section 6.3) views can be launched using the **Show** option in the respective menu.

Manual Validation — The peptide table menu has the extra option **Manual Validation** (see section 6.4.3).

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Note The checkmarks in the **OK** column and flags in the protein and peptide parts of a table are completely independent of each other. This means that a peptide can be accepted (**OK** selected) even if the respective protein has not been accepted. Equally, an accepted protein hit can contain peptides that did not meet the acceptance criteria (**OK** not selected).

6.2 Protein-Specific Views

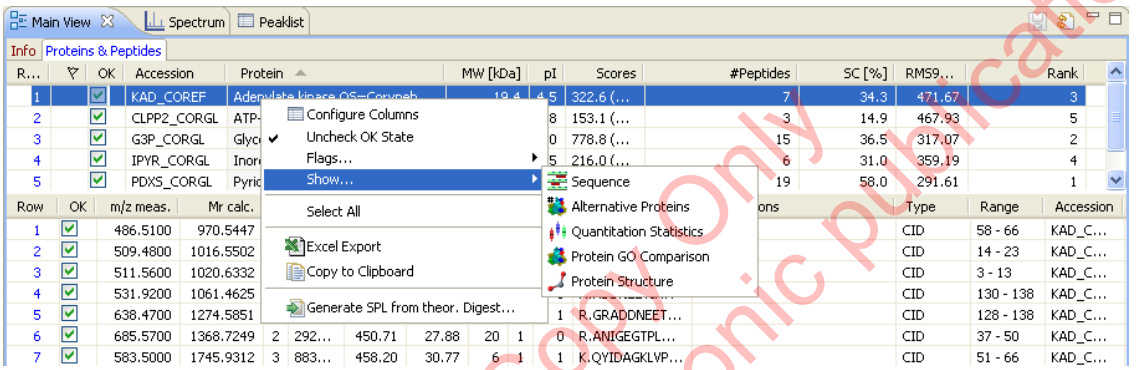

Figure 6-4 Views available through the Protein Table shortcut menu

Three Views can be launched from the Protein Table menu (see Figure 6-4):

- Sequence View (see section 6.2.2)
- Alternative Proteins View (see section 6.2.3)
- Quantitation Statistics View (see section 7.1.4)
- Protein GO Comparison View (see section 6.2.4.1)
- Protein Structure View (see section 6.2.5)

6.2.1 Protein Info View

The Protein Info View displays a summary of protein database information (accessions, gene names, sequence, and so on) for a protein selected in the protein table.

The Protein Info View is opened by selecting a protein in the protein table and selecting **Window > Show View > Protein Info**.

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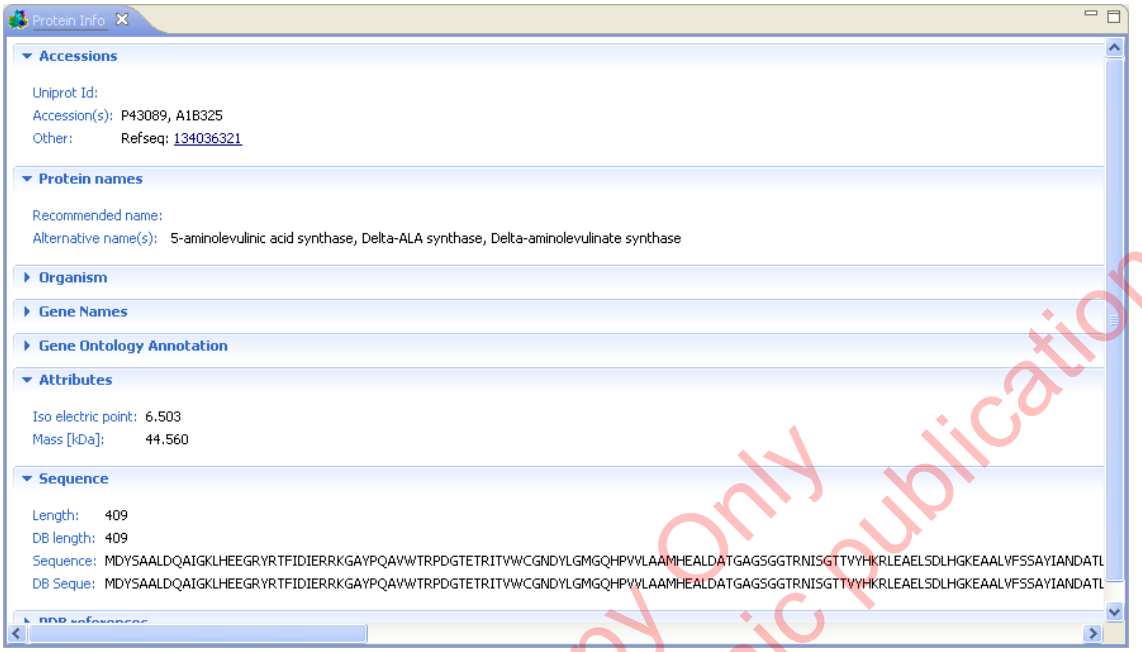

Figure 6-5 Protein Info View

6.2.2 Sequence View

The **Sequence View** is opened through the Protein Table menu (**Show > Sequence**) or the main menu (**Window > Show View > Sequence**).

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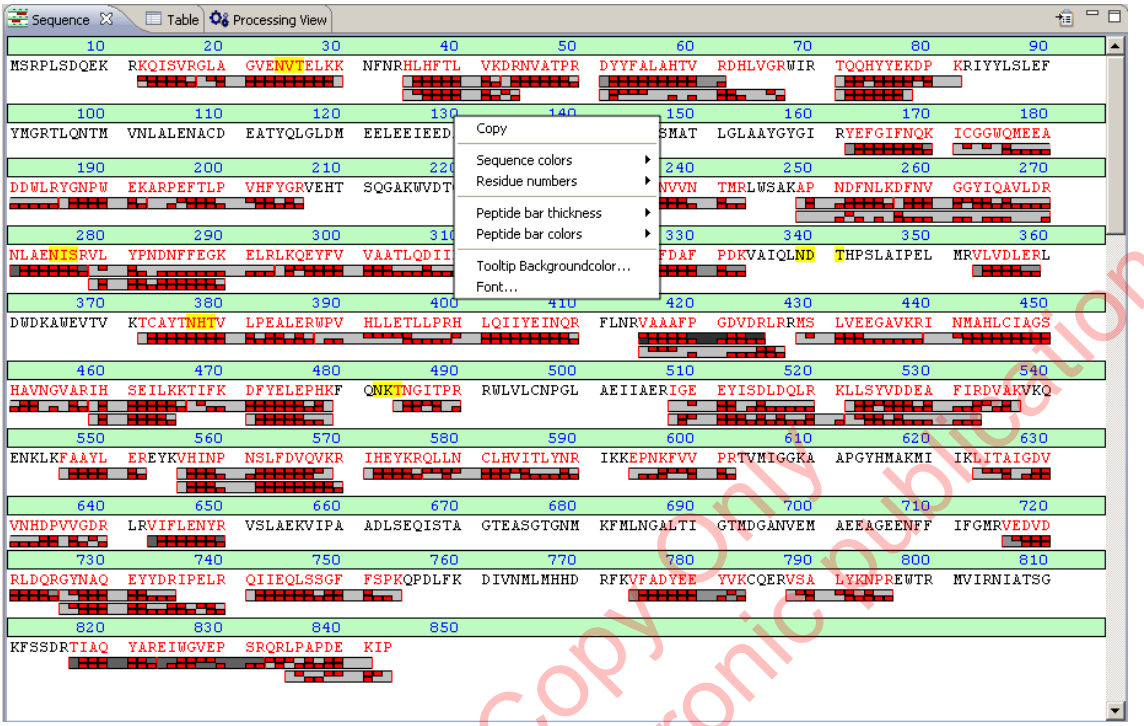

Figure 6-6 Sequence View with shortcut menu

The **Sequence View** displays a sequence coverage map of the protein selected in the Protein Table.

Right-clicking the **Sequence View** opens a shortcut menu that allows users to define a color scheme for the map (see Figure 6-6).

Table 6-3 Sequence View toolbar buttons

Button	Name	Action
	Copy to Clipboard	Copies the contents of the selected protein's **Accession**, **Protein** and **Sequence** columns to the clipboard. The protein sequence can also be copied as text from the **Sequence** column of the protein part of the Proteins&Peptides table.

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6.2.2.1 Sequence View Shortcut Menu

The options in the **Sequence View** menu enable users to change the appearance of the sequence and its labels. Right-click anywhere in the **Sequence View** to open the menu.

Copy

Enables the map to be pasted into other applications (for example, Word, PowerPoint).

Sequence Colors

Change the appearance of the **Sequence View**.

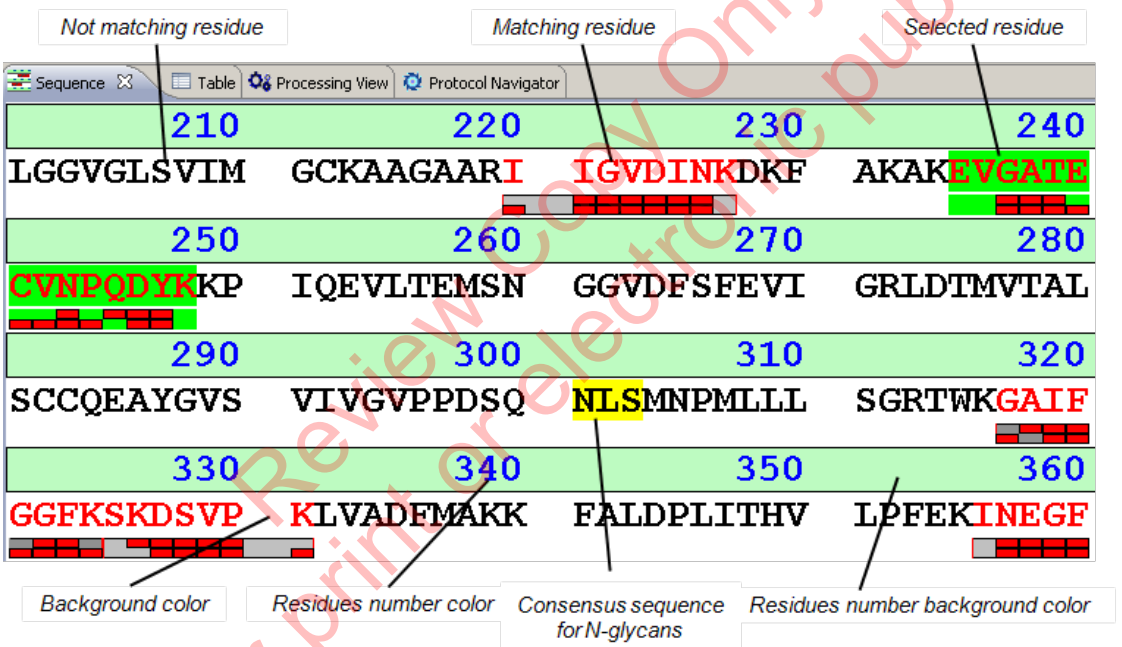

Figure 6-7 Coloring scheme of the Sequence View

- Color of matching residues
- Color of not matching residues
- Color of selected residues (residues belonging to the peptide selected in the peptide table)

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- Color of N-Glycans (potential sites of N-glycosylation)
- Background color

Selecting any of the options above opens the **Color** dialog box.

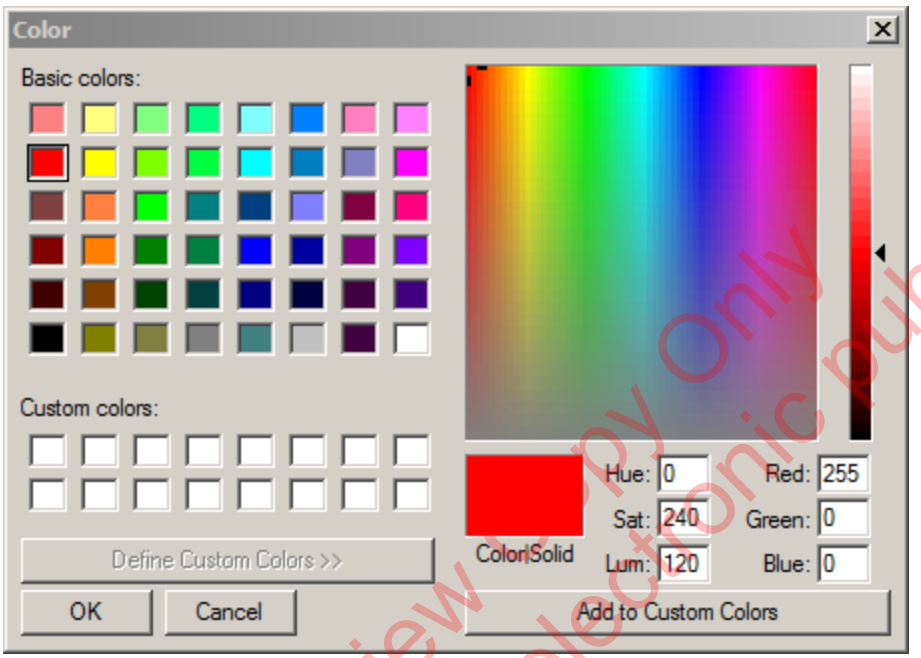

Figure 6-8 Color dialog

Residue numbers

Change the appearance of the residue number bar above the sequence.

- Show numbers (switch on and off)
- Residues number background color
- Residues number color

Peptide bar thickness

Change the appearance of the peptide bar below the sequence by selecting the desired option. The active option is indicated by a check mark.

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- No
- Thin
- Medium (default)
- Thick

Peptide bar colors

Change the appearance of the peptide bar below the sequence by selecting the desired option. The active option is indicated by a check mark.

- Peak intensity (default)
- MS/MS score
- Peak Quality Factor

ToolTip Background Color

Define the background color of the ToolTip window.

Font

Define the font, appearance and size of the text in the **Sequence** View.

6.2.2.2 Sequence Map

The sequence map consists of gray bars that indicate identified peptides. The relative peak intensity, MS/MS score or Peak Quality Factor is indicated by the darkness of the shading; the darker the shading, the higher the respective value.

For peptides identified by MS/MS, the sequence boundaries of fragment ions that have been found and support the corresponding amino acid sequence are indicated by red vertical separators in the gray peptide bars (see Figure 6-9).

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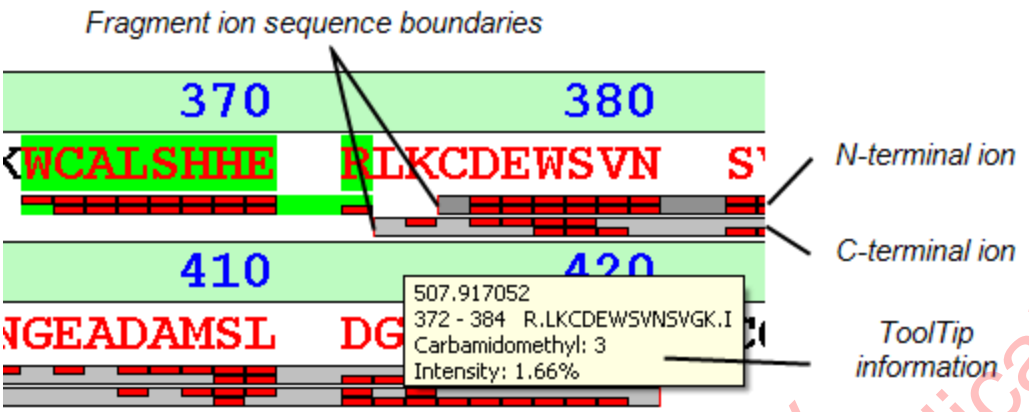

Figure 6-9 Sequence map information

Where two gray peptide bars lie above one another, the upper bar represents an N-terminal ion (CID: B, B-17. ETD: C) while the lower bar represents a C-terminal ion (CID: Y. ETD: Z+1, Z+2). Where only one bar is shown, it represents the N-terminal ion.

Clicking a gray bar highlights the residues in the sequence map (color = **Selected residue**), loads the respective spectrum into the **Spectrum** View, and highlights the respective peptide in the Peptide Table.

Hovering the mouse pointer over a peptide launches a ToolTip that displays the **calculated *m/z***, **Range**, **Sequence**, **Modifications** and **Peak Intensity** values of the respective peptide in the Peptide Table.

6.2.3 Alternative Proteins View

The Alternative Proteins View is opened through the Protein Table menu (**Show > Alternative Proteins**) or the main menu (**Window > Show View > Alternative Proteins**).

Protein databases frequently contain a number of similar entries — homologous proteins from different species, splicing variants, proteins including or excluding signal peptides, and so on.

This means that a whole group of proteins may be identified for a given data sets. In such cases, the Protein Table displays the best hit, that is, the hit with the highest score. The other, similar proteins can be accessed in the **Alternative Proteins** View.

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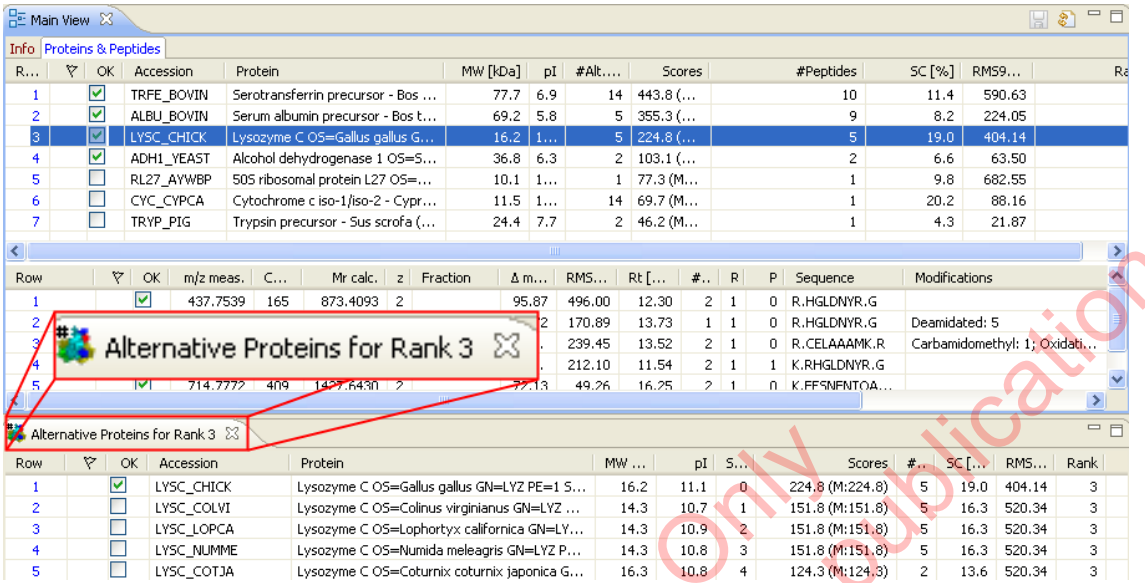

Figure 6-10 Alternative Proteins View

For a given protein, the **Alternative Proteins** View shows all equivalent matches that cannot be discriminated on the basis of the underlying MS data and subset matches that contain a subset of the peptides of the best hit.

In essence, the **Alternative Proteins** View is a second Protein and Peptides table (see section 6.1.2.1) with the same parameters and functionality. To display the sequence map for a protein in the **Alternative Proteins** View, launch the shortcut menu by right-clicking a protein and select **Show > Sequence**.

Each protein in the Alternative Proteins Protein Table can be selected in the **OK** column. All selected proteins will appear in the Protein table. If all check marks are cleared, the best hit of the protein group will be shown in the Protein table.

To see a different protein in the Protein Browser, change the check marks manually. All proteins that are selected in the Protein Selector also appear in the Protein table.

6.2.4 Protein Gene Ontology (GO) View

The Protein GO View displays a gene ontology diagram for a protein selected in the protein table.

The Protein GO View is opened by selecting a protein in the protein table and selecting **Window > Show View > Protein GO**.

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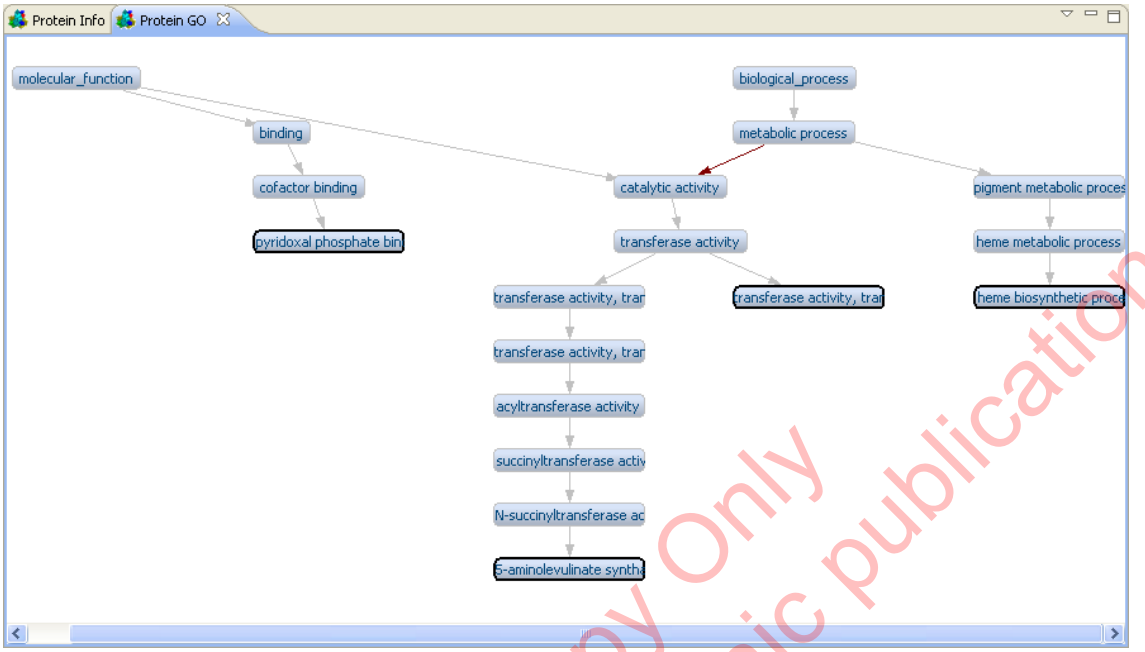

Figure 6-11 Protein GO View tree diagram

Table 6-4 Protein GO View toolbar buttons

Button	Name	ToolTip	Action
	View Menu	View Menu	Displays a list of ontology diagram types: select from **Tree**, **Spring**, **Radial** and **Grid**.

Table 6-5 Protein GO View shortcut menu commands

Shortcut menu command	Action
Copy to Clipboard	Copies the current image to the clipboard.
Save	Saves the current image as bmp, jpg or png file.

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6.2.4.1 Protein Gene Ontology (GO) Comparison View

The Protein GO Comparison View enables ontological comparison of two or more proteins selected in the protein table.

The Protein GO Comparison View is opened by selecting two or more proteins in the protein table, right-clicking and selecting **Show > Protein GO Comparison**.

The results are displayed in tabular or pie-chart format. The **Table** page displays a grid showing the ontological classification(s) that apply to the respective protein.

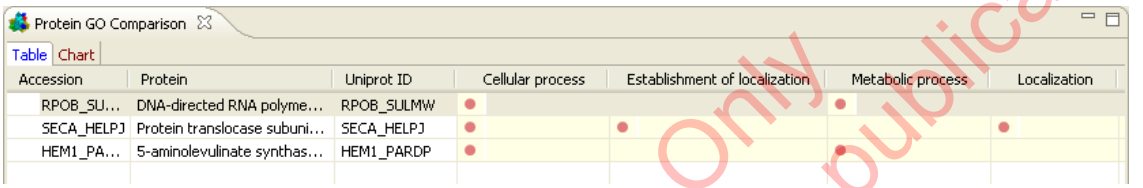

Table 6-6 Protein GO Comparison View Table page

The **Chart** page (see Figure 6-12) displays the same information in pie-chart form. The pie slices indicate the number of compared proteins associated with the respective ontological classification. The classifications are divided into three groups; biological processes, cellular components and molecular functions. Select a button in the toolbar to display the pie-chart for the respective group.

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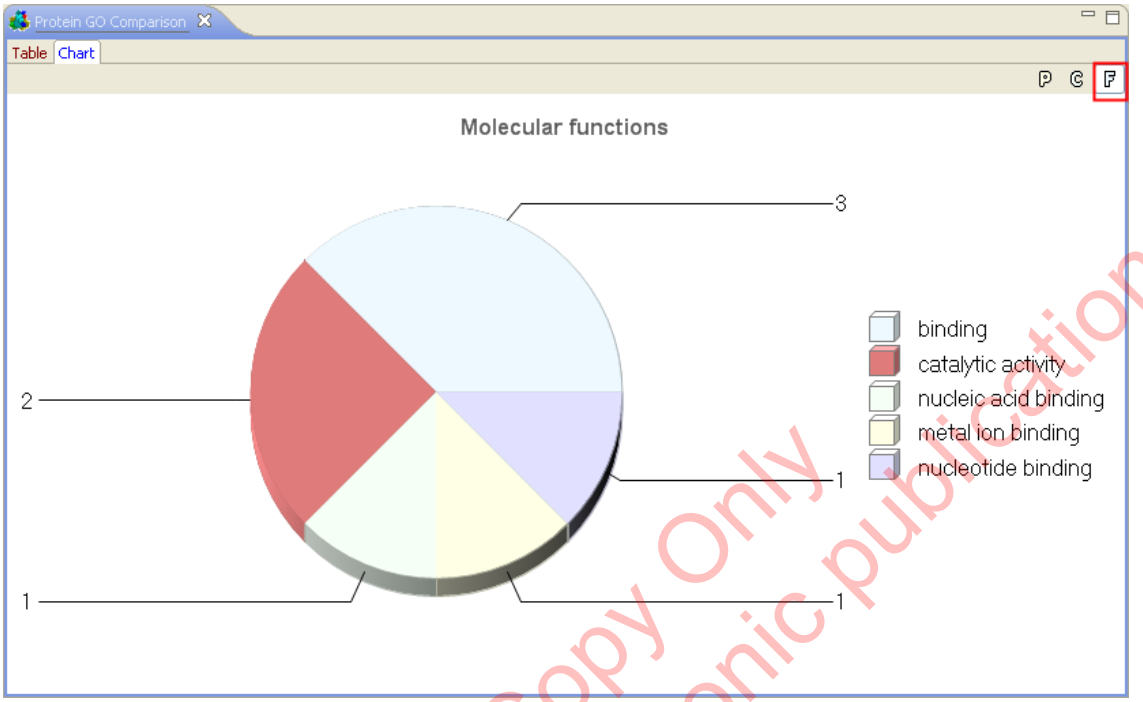

Figure 6-12 **Protein GO Comparison View Chart page displaying molecular functions associated with the compared proteins**

Table 6-7 **Protein GO Comparison Chart page toolbar buttons**

Button	Name	Action
P	**Biological processes**	Displays pie-chart showing number of compared proteins associated with the respective biological process.
C	**Cellular components**	Displays pie-chart showing number of compared proteins associated with the respective cellular component.
F	**Molecular functions**	Displays pie-chart showing number of compared proteins associated with the respective molecular function.

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Table 6-8 Protein GO Comparison View shortcut menu commands

Shortcut menu command	Action
Copy to Clipboard	Copies the current image to the clipboard.
Save	Saves the current image as svg or png file.

6.2.5 Three-dimensional (3D) Structure View

The 3D Structure View displays the PDB three-dimensional structure (if available) of a protein selected in the protein table .

By default, identified peptides are displayed in blue and modified amino acids in red. This enables easy validation of the plausibility of a peptide match. For example, it would be expected that a phosphorylation is located at the surface of a protein and one would not expect to find many identified peptides in the transmembrane regions of a membrane protein.

The correct representation of identified peptides and modified amino acids is based on residue numbers. If the sequence of the identified protein is not identical with the sequence of the protein in the PDB file, a sequence offset must be applied. ProteinScape tries to find the correct offset automatically.

The 3D Structure View is opened by selecting a protein in the protein table, right-clicking and selecting **Show > Protein Structure**.

The structure can be rotated by clicking and dragging in the View.

Zoom in and out using the mouse wheel or pressing **SHIFT** and clicking and dragging.

Pressing **SHIFT** and double-clicking and dragging enables translation of the image in the x- and y-directions.

A shortcut menu enables the representation to be changed, for instance, to a surface or sketch representation. Alternatively, the representation can be changed by typing commands into the 3D Structure Console (see section 6.2.5.2).

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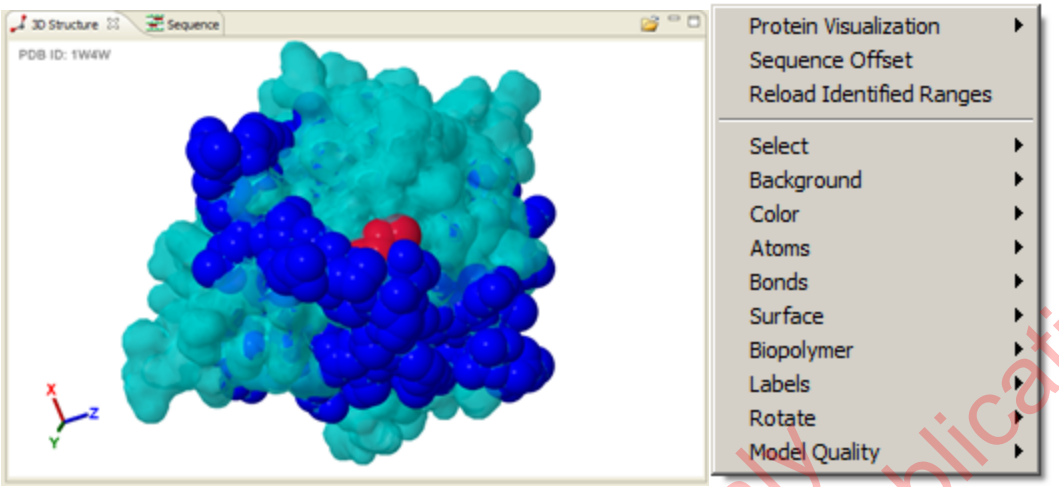

Figure 6-13 3D Structure View with shortcut menu

Table 6-9 3D Structure View toolbar buttons

Button	Name	Action
	Open	Opens the Open PDB-File dialog box for opening a PDB file that has been stored on the client computer's local hard disk.

6.2.5.1 Changing the 3D Structure View Representation

The 3D Structure View can display the 3D structure of the selected protein in four predefined representations. The desired representation can be chosen from options under **Protein Visualization** in the view shortcut menu.

- **Surface** displays a space-filling model of the protein's solvent-accessible surface.
- **Sketch** displays a cartoon of alpha helices and beta sheets.
- **Atoms** displays a ball-and-stick model with the atoms in a uniform color.
- **Default** displays a ball-and-stick model with the atoms colored using standard chemical color coding.

The offset used for the representation of identified peptides and modified amino acids can be changed manually using the shortcut menu **Sequence Offset** command. After applying a new sequence offset, select **Reload Identified Ranges** to apply the new offset.

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For some proteins, several PDB files are available. ProteinScape selects the file with the largest sequence range and the lowest number of chains. Any of the avaliable PDB files can be loaded in the 3D Structure View using the respective links in the PDB references section of the Protein Information View. After loading a file, select **Reload Identified Ranges** to display identified peptides and modified amino acids.

6.2.5.2 Three-dimensional (3D) Structure Console View

The 3D Structure View Console View enables manipulation of the structural representation in the 3D Structure View using the Jmol command language. A list of commands can be found at http://chemapps.stolaf.edu/jmol/docs/.

The 3D Structure View Console is opened by selecting **Window > Show View > 3D Structure Console**.

6.3 Peptide-Specific Views

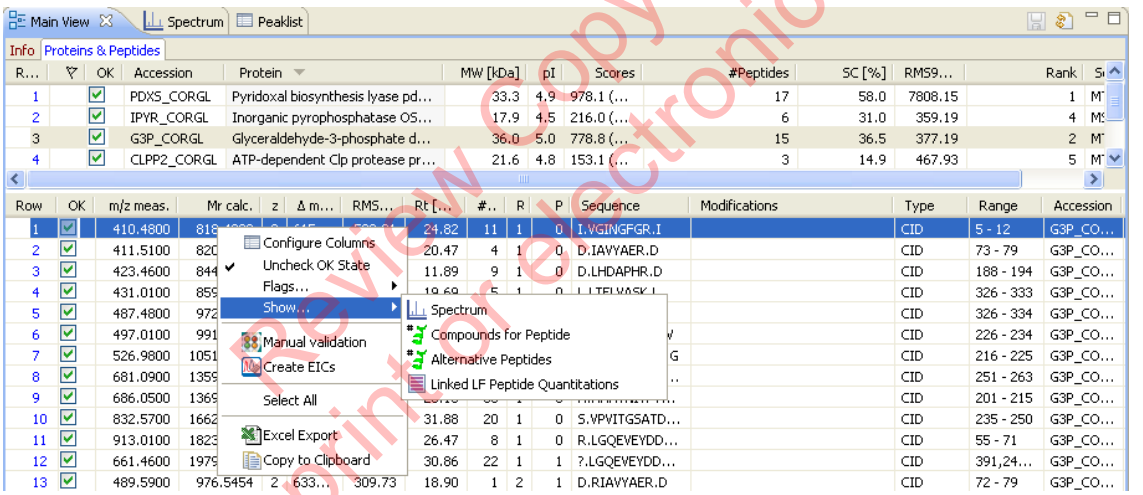

Figure 6-14 Views available through the Peptide Table shortcut menu

Three Views can be launched from the Peptide Table menu (see Figure 6-14).

- Spectrum View (see section 6.4.2)
- Compound for Peptide View (see section 6.3.2)
- Alternative Peptides View (see section 6.3.3)

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6.3.1 Peaklist View

The Peaklist View is opened through the Spectrum View shortcut menu (**Show Peak list**) or the main menu (**Window > Show View > Other > Peaklist**).

The Peaklist View displays the peak list of the selected peptide or glycan in a Peptide or Glycan table. The Peaklist View of an MS spectrum also displays WARP workflow criteria and calibrant list information (see section 5.6.2).

The peak list can be copied to the clipboard or exported to Microsoft Excel using the respective options in the shortcut menu.

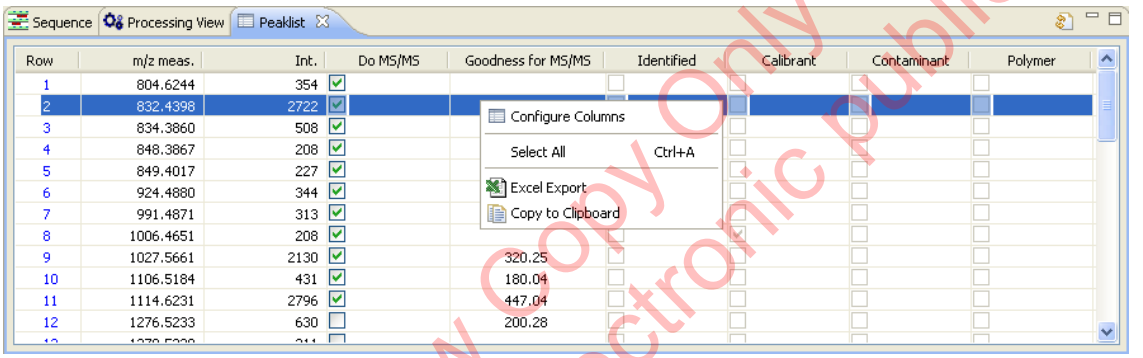

Figure 6-15 Peaklist View of an MS spectrum with shortcut menu

6.3.2 Compounds for Peptide View

The **Compounds for Peptide** View is opened through the Peptide Table menu (**Show > Compounds for Peptide**).

For a given protein, there may be a number of compounds in the LC-MS/MS data sets that match a particular peptide in the sequence. This is reflected in the **#Cmpds.** column of the Peptide table (see Figure 6-16).

The Peptide table displays only one compound per peptide, that is, the compound with the highest ion score.

Additional compounds with lower ion scores can be listed in the **Compounds for Peptide** View.

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In essence, the **Compounds for Peptide** View is a second Peptides table with virtually the same parameters and functionality (the **Manual Validation** menu option is not available).

Selecting or clearing the **OK** box of a compound in the **Compounds for Peptide View** table adds it to or removes it from the Peptide table immediately above.

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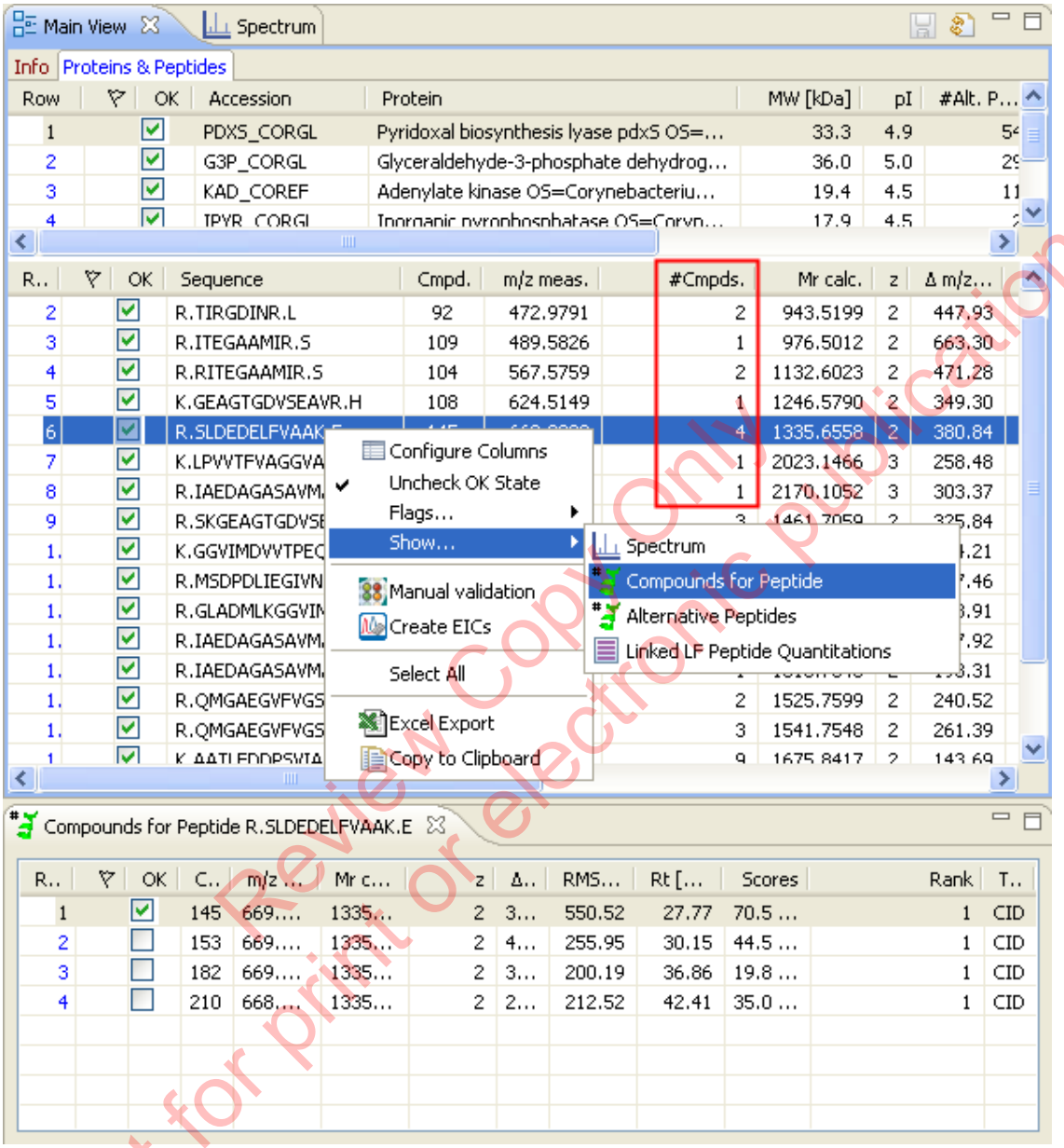

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Figure 6-16 Compounds for Peptide View displays additional compounds for the selected peptide

6.3.3 Alternative Peptides View

The **Alternative Peptides** View is opened through the Peptide Table menu (**Show > Alternative Peptides**) or the main menu (**Window > Show View > Alternative Peptides**).

The Peptide table usually displays only one of all possible matches. However, there are usually several possible matching peptide sequences for a given compound. They are sorted according to ion score and given a **Rank** number — with 1 being the best match.

The Alternative Peptides table shows all alternative matches to a given Compound that belong to the same protein or to another protein in the list. This is particularly useful in the case of a modified peptide with more than one possible modification site. Here, it is often difficult to decide which site is actually modified. The Alternative Peptides table gives access to all possible matches, and in combination with the Spectrum View, enables detailed manual validation (see section 6.4.3).

6.4 Data Views

Four data-based Views are available in ProteinScape.

- **LC-MS Survey** View
- **Gel** View
- **Processing** View
- **Progress** View

6.4.1 Viewing Single MS and MS/MS Spectra

Single MS and MS/MS spectra can be Viewed in the **Spectrum** View (see section 6.4.2). Note that the ProteinScape database stores spectra at the peak list level. Raw spectrum data are available through the external program BioTools.

The **Data Info** area of the data set's Main View displays information about the spectrum.

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Table 6-10 Data set main View parameters

Parameter	Comments
Name	Name of the spectrum.
Owner	The user who imported or exported the spectrum to ProteinScape.
Source	The complete path to the spectrum, usually to the file `1r` or `peaklist.xml`. This is the path that will be transferred to BioTools. If the raw data is moved or the spectrum has been imported into ProteinScape from another PC, this path must be changed manually. BioTools can only access the raw data if this path is correct.
Mass Spec	The name of the MS instrument specified either in the raw data or manually during the interactive MS data import.
Date acquired/imported	The acquisition date specified in the raw data and the date of import into ProteinScape.
Note	Free text field for additional information.
Type	Type of fragmentation (for example, CID). MS/MS data sets only.
Precursor Mass	Mass of the MS/MS precursor ion. MS/MS data sets only.
Charge	Charge of the MS/MS precursor ion. MS/MS data sets only.

The **Peaklist** View (see section 6.3.1) associated with each spectrum shows the MS or MS/MS peak list (*m/z* and intensity).

6.4.2 Spectrum View

The **Spectrum** View is opened through the Peptide Table menu (**Show > Spectrum**) or the main menu (**Window > Show View > Spectrum**).

The **Spectrum** View displays an annotated histogram with *m/z* along the x-axis and intensity along the y-axis. A compound comment is displayed below the spectrum.

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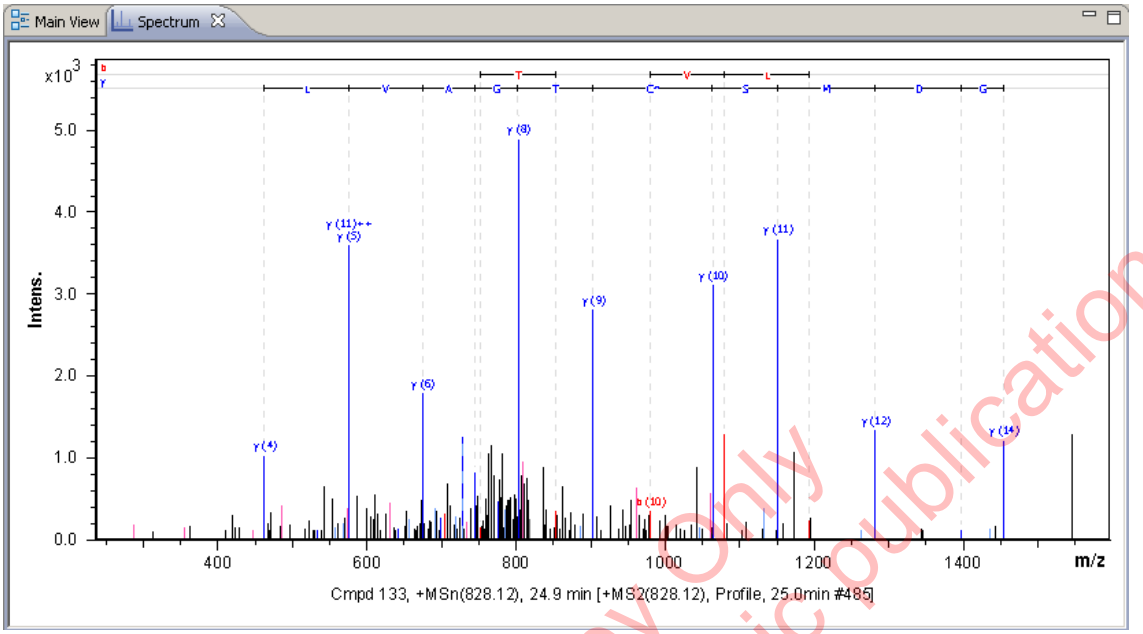

Figure 6-17 Spectrum View

The spectrum of a given peptide can be accessed in a number of ways:

- Clicking the respective entry in the peptide table (see section 6.1.2.2)
- Clicking the respective compound in the **Project Navigator** tree (see section 3.2)
- Clicking the respective gray bar in the sequence map (see section 6.2.2.2)
- in the LC-MS Survey View through single compounds.

Accessing spectra in the ways described above displays a single MS or MS/MS spectrum. To display the MS spectrum together with the MS/MS spectrum in a split window, use an XML file from DataAnalysis containing some MS peaks in addition to the precursor or use an LC-MALDI dataset transferred to ProteinScape by WARP-LC.

6.4.2.1 Spectrum View Shortcut Menu

The **Spectrum** View menu enables the appearance and annotation of a peptide spectrum to be defined.

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- **Show Masses** — Show/Hide mass labels above peaks
- **Distance** — Activate/Deactivate **Distance** tool. When the **Distance** tool is activated, left-click and drag on the spectrum to display the distance in the x-direction between two peaks. When the **Distance** tool is deactivated, clicking and dragging creates a red-framed zoom area (see Figure 6-18). Double-clicking anywhere in the spectrum resets the View.

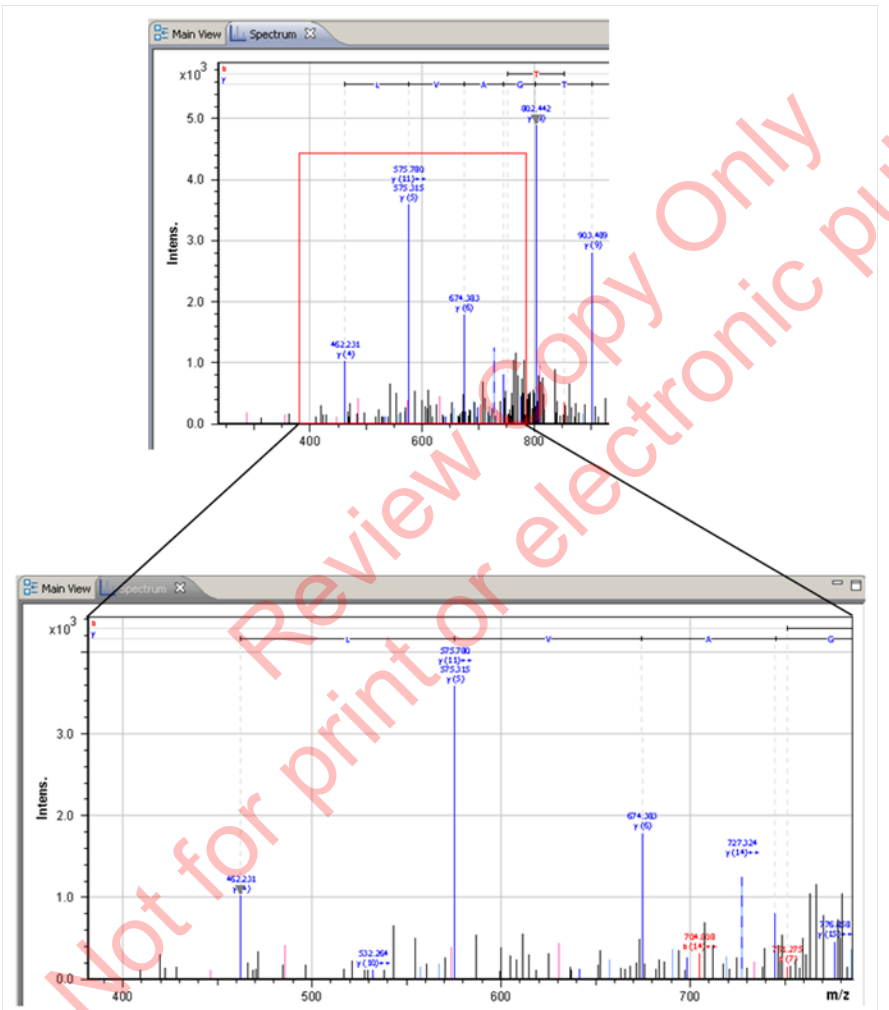

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Figure 6-18 Zooming in on a selected area in the Spectrum View

- **Select Spectrum** — Select spectrum displayed.
- **Copy to Clipboard** — Enables the annotated spectrum to be pasted into other applications (for example, Word, PowerPoint).
- **Show Report** — Generate a detailed report in the selected format.
- **Preferences** — Define peak and annotation colors used to display ion series (see Figure 6-19).
  - **Peak Line Width** — Select the desired width of peak lines (pixels)
  - **Image Size Percentage** — Used to display glycan structures (see section 8.6.4.4)
  - **Magnified Image Size Percentage** — Used to display glycan structures (see section 8.6.4.4)
  - **Image Layout** — Used to display glycan structures (see section 8.6.4.4)

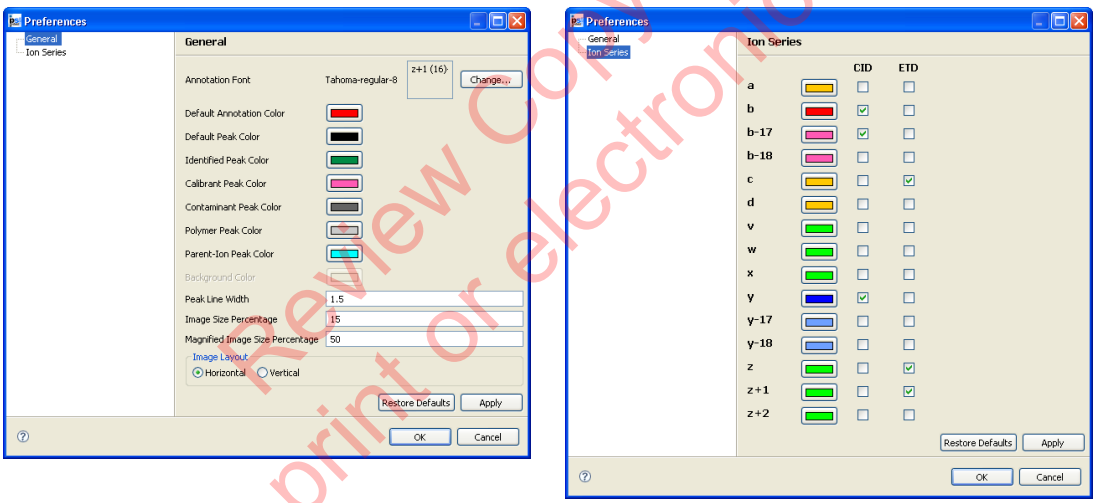

Figure 6-19 General (left) and Ion Series (right) Preferences dialogs

6.4.2.2 Changing Axis Displays

To change the y-axis display:

- Hover the mouse pointer to the left of the y-axis. The mouse pointer arrow will change to a double-headed vertical arrow ↕.

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- Click and hold the left mouse button to scroll along the axis.
- Click and hold the right mouse button to stretch or contract the axis.

To change the x-axis display:

- Hover the mouse pointer below the x-axis. The mouse pointer arrow will change to a double-headed horizontal arrow ↔.
- Click and hold the left mouse button to scroll along the axis.
- Click and hold the right mouse button to stretch or contract the axis.

Double-clicking anywhere in the spectrum or selecting the respective **y/x-axis autoscale control** check box in the lower left corner of the View resets the corresponding axis.

6.4.3 Manually Validating Data Using BioTools

The external program BioTools can be used for manual validation of peak list data.

►► To transfer peak list data to BioTools

- **Single MS spectrum** (MALDI PMF file) or **LC-MS/MS run** — Click **Manual validation** in the **Search Result Info** area of **Info** page of the **Search Result Main View**. All accepted proteins and all peptides and associated MS/MS spectra (if available) are opened in BioTools .

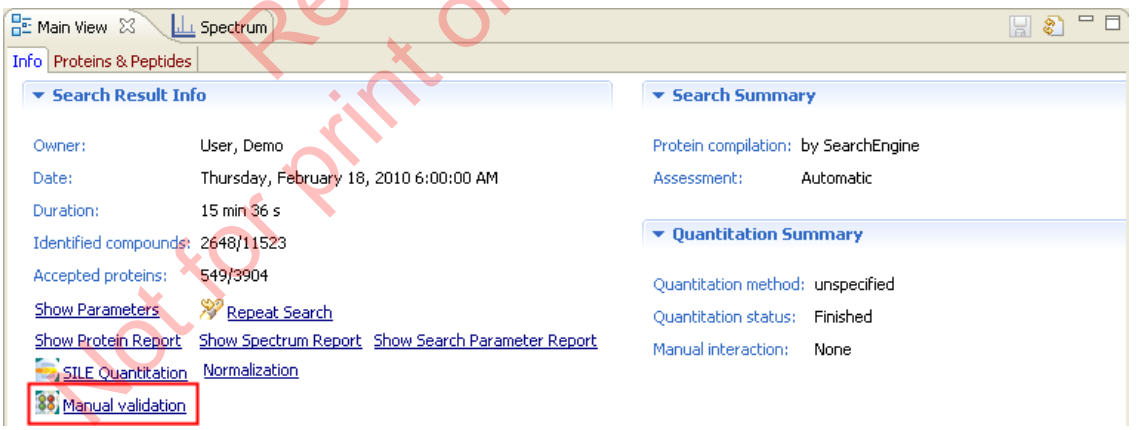

Figure 6-20 Manual validation option in the Search Result Info area of Info page of the Search Result Main View

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- **Single MS/MS spectrum** — Right-click
  - a compound in the Project Navigator tree or
  - a peptide in the Proteins & Peptide Table

  and select **Manual Validation** from the shortcut menu. The associated MS/MS spectrum is loaded into BioTools and displayed with the respective peptide match.

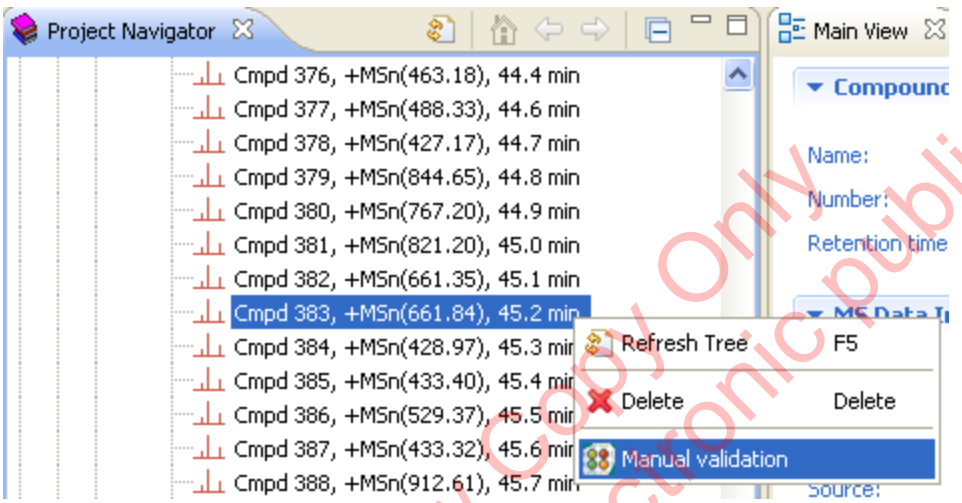

Figure 6-21 Manual validation command in the shortcut menu of the Project Navigator

Editing peak lists in BioTools

Peak lists can be edited in BioTools through the shortcut menu (**Add** / **Remove Peak** commands) or the **Analysis** menu.

Peak lists are transferred back to ProteinScape by clicking the button and the edited peak lists are used in subsequent searches. The original peak lists are stored in the ProteinScape database.

6.4.4 LC-MS Survey View

The **LC-MS Survey** View is opened through the main menu (**Window > Show View > Gel**). It provides an overview of the entire LC-MS/MS run.

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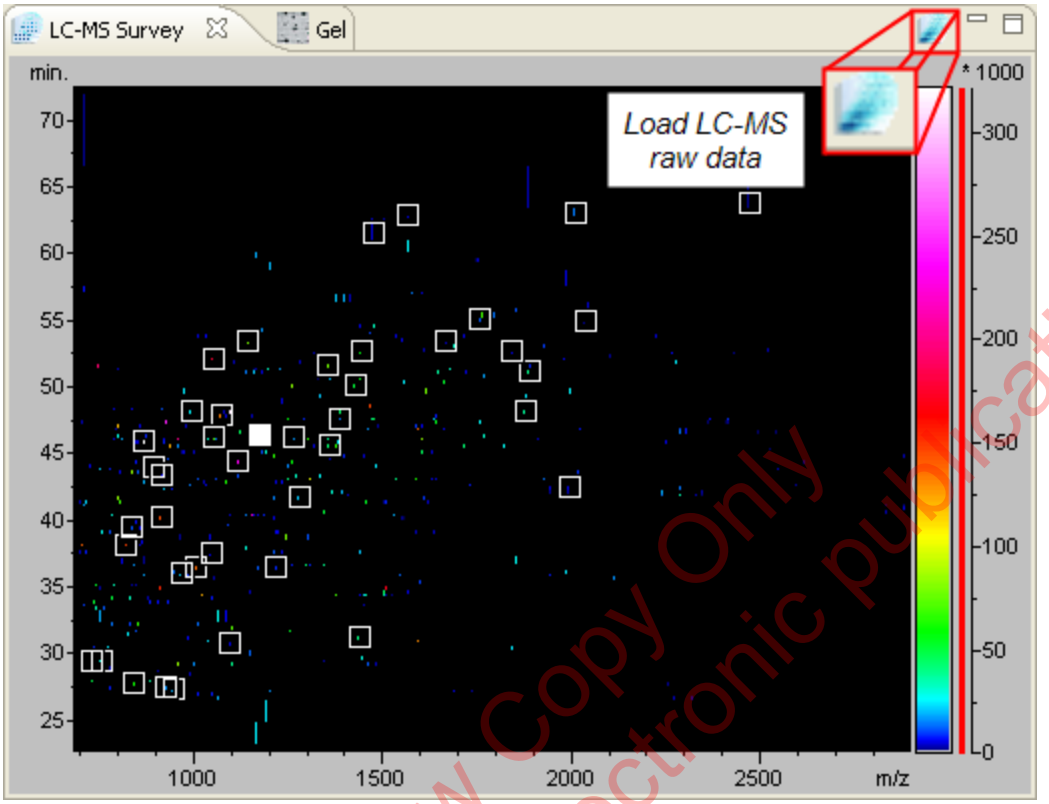

Figure 6-22 LC-MS Survey View

LC-MS raw data can be loaded into the LC-MS View by clicking the symbol in the LC-MS Survey View toolbar.

To load raw data, the raw data file location (= **Source** on the MS or LC-MS/MS data sets Main View) must be correct. If the data path is incorrect, it must be adjusted (see section 4.1.4).

Very large data sets can be filtered to prevent overloading the client. To set filter criteria, right-click in the **LC-MS Survey** View and select the desired option from the shortcut menu.

6.4.4.1 LC-MS Survey View Shortcut Menus

The **LC-MS Survey** View contains data and axis menus.

Right-click anywhere within the data display to access the window menu.

Right-click anywhere on the respective axis to access the axis menu.

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LC-MS Survey View Data Shortcut Menu

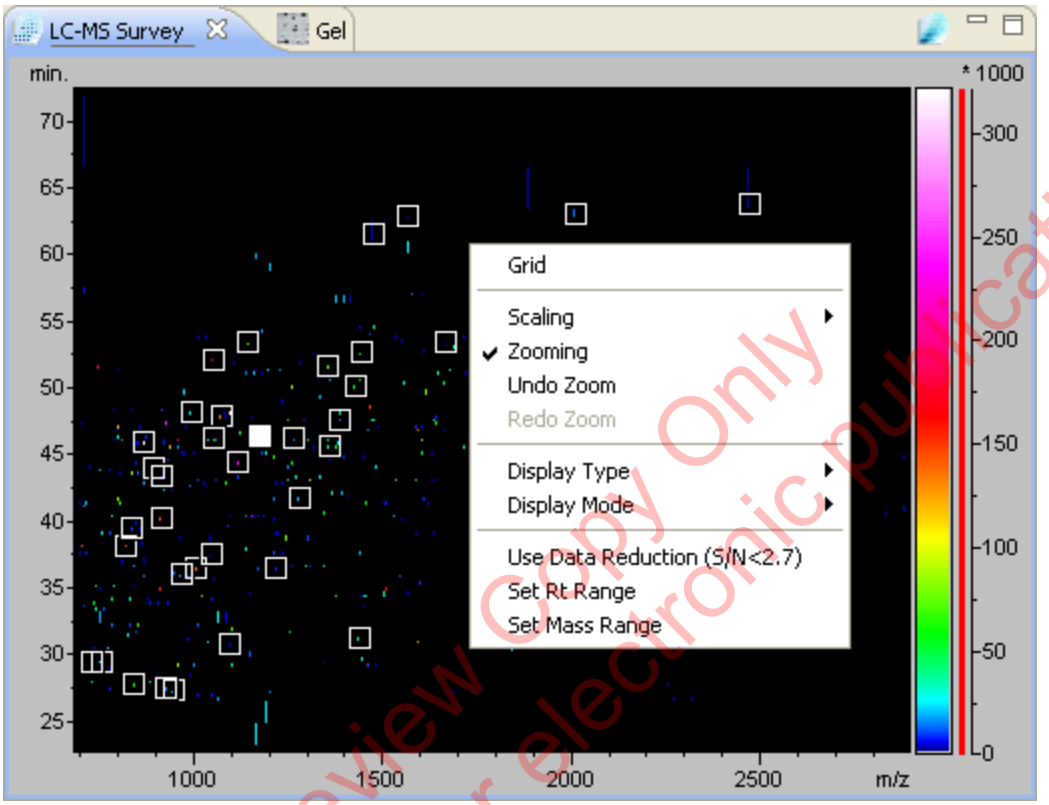

Figure 6-23 LC-MS Survey View shortcut menu

Grid — Show/Hide grid on window

Scaling — **Expand Manually** opens the Manual Scaling dialog box for setting axis maximum and minimum values. Select **Reset** to reapply default values.

Zooming — Enable/Disable zoom using click and drag. Note that the View can be zoomed using the mouse wheel even when **Zooming** is disabled.

Undo/Redo Zoom — Navigate through a zoom sequence

Display Type — Choose the desired type of diagram (see section 6.4.4.2)

Display Mode — Set the LC-MS Survey View display mode.

Use Data Reduction (S/N<2.7) — Toggle using data reduction filter during import

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Set Rt Range — Opens the **Set Rt Range to Load** dialog box for setting start and end retention times. Enter –1 into the lower field to import all retention times.

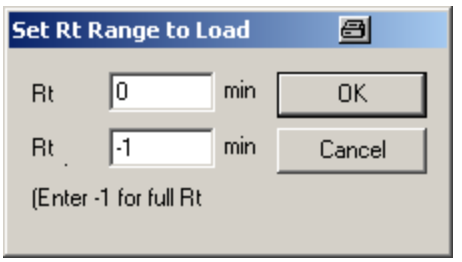

Figure 6-24 Set Rt Range to Load dialog

Set Mass Range — Opens the **Set Mass Range to Load** dialog box for setting minimum and maximum *m/z* values. Enter –1 into the lower field to import all m/z values.

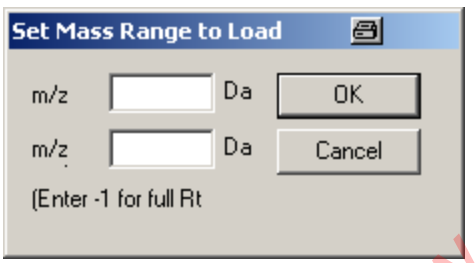

Figure 6-25 Set Mass Range to Load dialog

LC-MS Survey View Axis Shortcut Menu

Changing the y-axis display

- Hover the mouse pointer to the left of the y-axis. The mouse pointer will change to a double-headed vertical arrow ↕.
- Click and hold the left mouse button to scroll along the axis.
- Click and hold the right mouse button to stretch or contract the axis.

Changing the x-axis display

- Hover the mouse pointer below the x-axis. The mouse pointer will change to a double-headed horizontal arrow ↔.

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- Click and hold the left mouse button to scroll along the axis.
- Click and hold the right mouse button to stretch or contract the axis.

Double-clicking anywhere in the window resets the View.

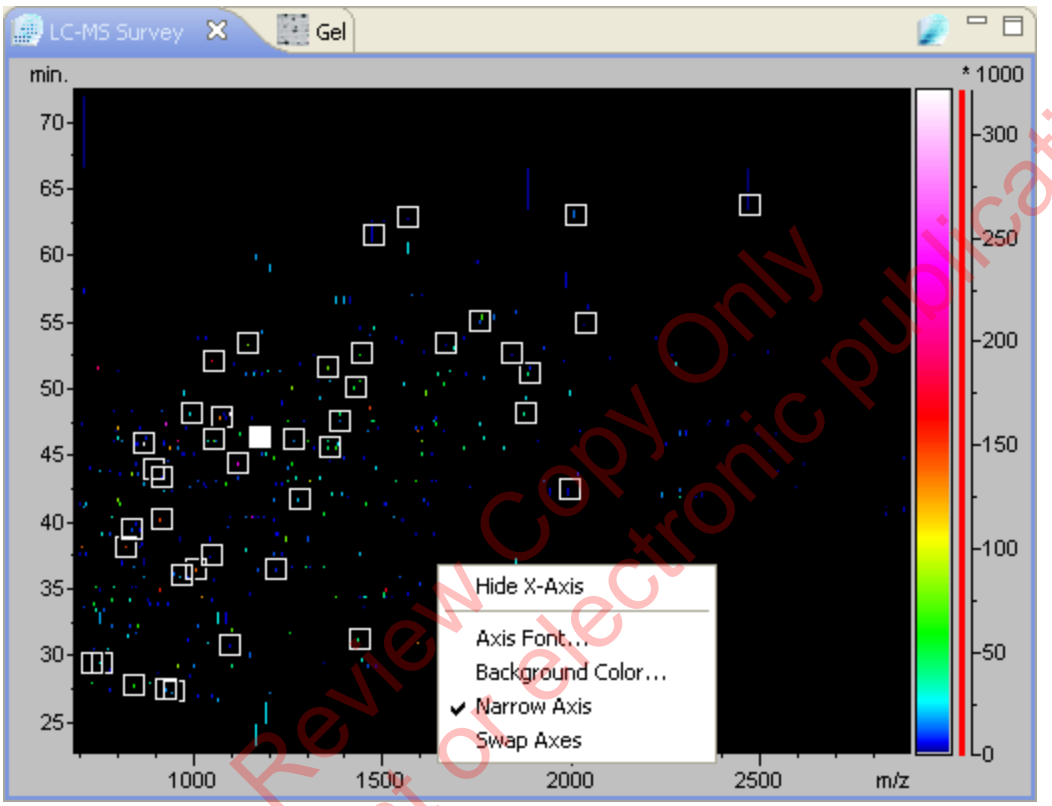

Figure 6-26 LC-MS Survey View Axis Shortcut Menu

Show/Hide Y/X-Axis — Toggle display of Y/X-axis. To show hidden axis, move mouse pointer to left/bottom edge of View and right-click to launch menu.

Axis Font — Define the font, appearance and size of the axis labels

Background Color — Define the background color of the View frame

Narrow Axis — Toggle narrow/wide View frame

Swap Axes — Swap x and y axis (default x = *m/z*, y = Rt)

Changing the color scale axis display

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The color scale in the 2D Density Plot has its own menu for choosing the type of color Scale (**Gray**, **Rainbow** (default), **Chromatic**, **Blue** or **Bipolar**) and the type of scale applied over the color axis (**Linear**, **Quadratic** or **Logarithmic**). These options are also available in the 2D Density Plot menu.

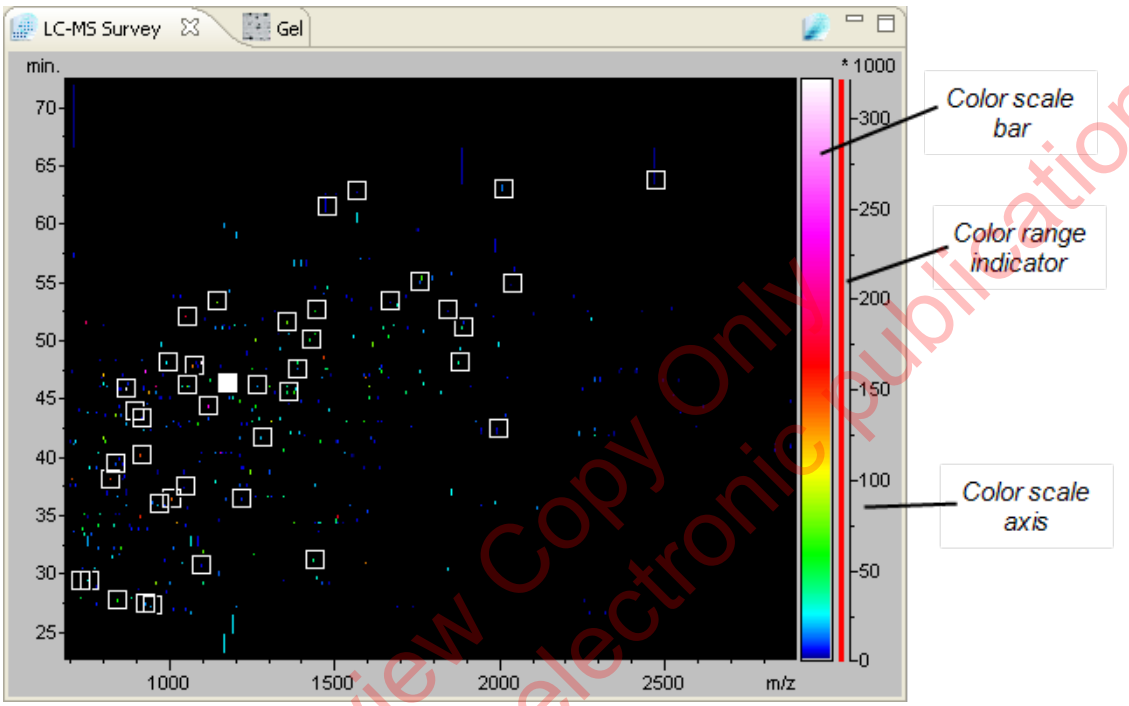

Figure 6-27 Color scale axis display

The range of colors used in the plot can be controlled by stretching or expanding the axis. The color range indicator shows the range of colors used in the plot (see Figure 6-27).

6.4.4.2 LC-MS Survey View Display Types

Data in the **LC-MS Survey** View can be Viewed as:

- a 2D Scan Plot,
- a 2D Density Plot,
- a 2D Stack Plot or
- a 2D All Scans Plot.

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2D Scan Plot

The 2D scan plot shows intensity plotted against *m/z*.

In addition to the functions contained in the window menu (see section 6.4.4.1), the 2D Scan Plot menu contains the following options:

Auto Scaling — Toggle automatic adjustment of scales to fit data

Display Mode — Select the type of plot (**Point**, **Line** or **Histogram**), the type of marker and the thickness of lines

2D Density Plot

The 2D density plot shows Rt plotted against *m/z*.

In addition to the functions contained in the View shortcut menu (see section 6.4.4.1), the 2D Density Plot menu contains the following options:

Display Mode — Select the type of color Scale (**Gray**, **Rainbow** (default), **Chromatic**, **Blue** or **Bipolar**) and the type of scale applied over the color axis (**Linear**, **Quadratic** or **Logarithmic**).

2D Stack Plot

The 2D stack plot displays Rt plotted against *m/z*.

In addition to the functions contained in the window menu (see section 6.4.4.1), the 2D Stack Plot menu contains the following options:

Background Color — Define the background color of the View

Spectrum Color — Define peak color

2D All Scans

The 2D All Scans display type shows intensity plotted against *m/z*.

In addition to the functions contained in the window menu (see section 6.4.4.1), the 2D All Scans Plot menu contains the following options:

Auto Scaling — Toggle automatic adjustment of scales to fit data

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Display Mode — Select the type of plot (Point, Line or Histogram), the type of marker and the thickness of lines

Background Color — Define the background color of the View

Spectrum Color — Define peak color

6.4.5 Gel View

The Gel View is opened through the main menu (**Window > Show View > Gel**).

Gel images are loaded into the Gel View by clicking a 1D-Gel or 2D-Gel node in the **Project Navigator** tree.

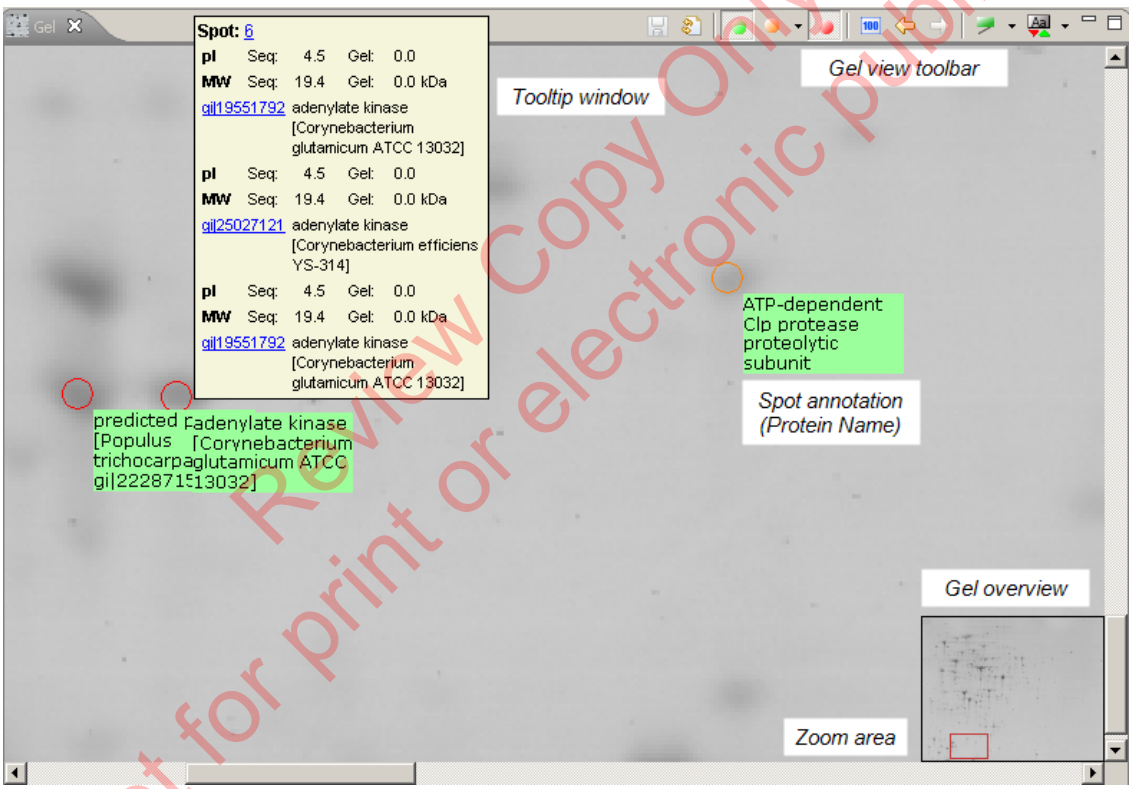
Gel
Spot: 6
pI Seq: 4.5 Gel: 0.0
MW Seq: 19.4 Gel: 0.0 kDa
gi|19551792 adenylate kinase [Corynebacterium glutamicum ATCC 13032]
pI Seq: 4.5 Gel: 0.0
MW Seq: 19.4 Gel: 0.0 kDa
gi|25027121 adenylate kinase [Corynebacterium efficiens YS-314]
pI Seq: 4.5 Gel: 0.0
MW Seq: 19.4 Gel: 0.0 kDa
gi|19551792 adenylate kinase [Corynebacterium glutamicum ATCC 13032]
Tooltip window
Gel view toolbar
ATP-dependent Clp protease proteolytic subunit
Spot annotation (Protein Name)
predicted [Populus trichocarpa gi|22287151
adenylate kinase [Corynebacterium glutamicum ATCC 13032]
Gel overview
Zoom area

Figure 6-28 Gel View displaying a 2D gel

6.4.5.1 Gel View Toolbar and Shortcut Menu

The toolbar in the upper-right corner of the Gel View is used to edit the gel image.

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Table 6-11 Gel View toolbar buttons

Button	Name	Action
	Save	Saves changes to the database.
	Refresh	Updates the View content from the database.
	Show Identified Spots	Displays identified spots in green circles.
	Show Spots in Progress	Displays spots with the status **Picked**, **Digested**, **Acquired**, and/or **Searched** (select status[es] from drop-down list) in orange circles.
	Show Detected Spots	Displays detected spots in green circles.
	Zoom Original	Reset View after zoom actions.
	Zoom Back	Go back one step in a sequence of zoom actions.
	Zoom Forward	Go forward one step in a sequence of zoom actions.
	Show Spot Annotations	Displays the selected spot annotation: select one from **None**, **Protein Name**, **Accession**, **Spot Memo** and **ToolTip Info**.
	Change Font Size	Displays spot annotations in the selected font size: select **6 pt**–**20 pt**.

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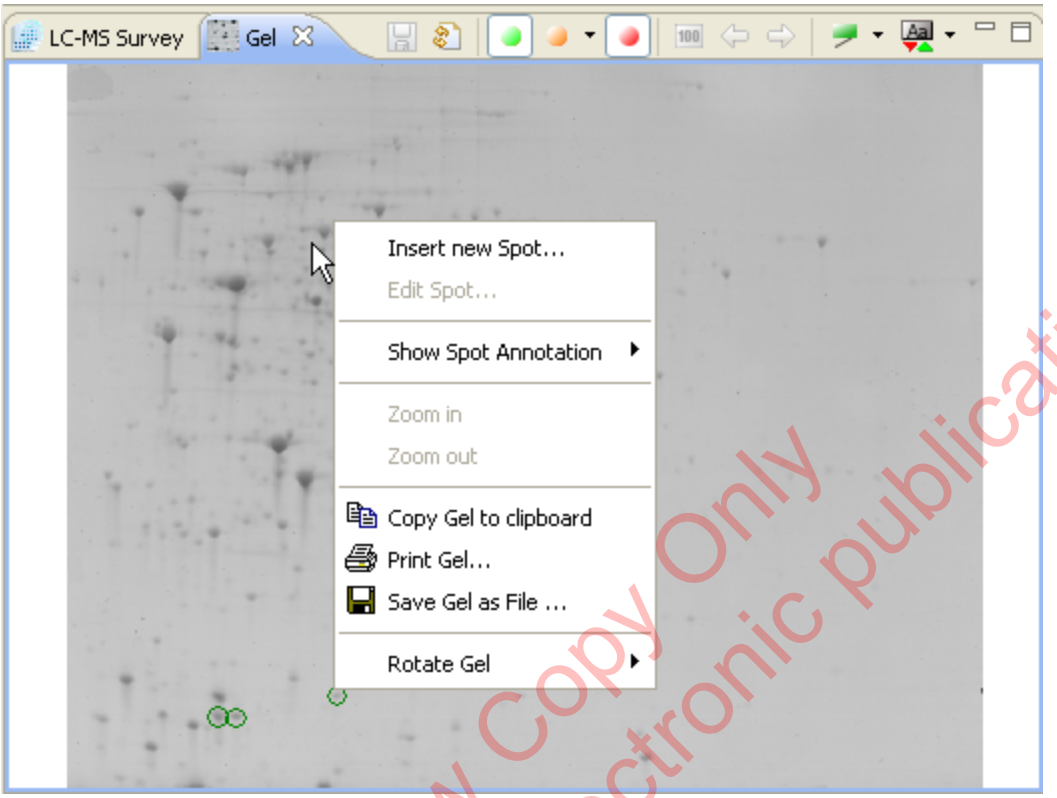

Figure 6-29 Gel View shortcut menu

Show spot annotations and the following additional functions can be accessed through the shortcut menu (right-click anywhere within the Gel View).

Insert new Spot — Opens the New Gel Spot dialog box (see section 3.5.2.3).

Edit Spot — Change the position of a spot by dragging and dropping. Only active when an existing spot is selected. Click **Save** in the toolbar to save changes in the database.

Show spot annotations — Select spot annotation content for individual spots. Options only active when an existing spot is selected.

Zoom in — Zoom in on gel

Zoom out — Zoom out from gel

Copy Gel to clipboard — Enables the annotated gel to be pasted into other applications (for example, Word, PowerPoint)

Print Gel — Send annotated gel image to a printer

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Save Gel as File — Save annotated gel as a graphic file

Rotate Gel— Rotate image 90° left or right, flip image horizontally/vertically

6.4.6 Processing View

The Processing View is opened through the main menu (**Window > Show View > Processing View**). It is recommended that the Processing View is opened in Fast View mode (see section 2.1.4).

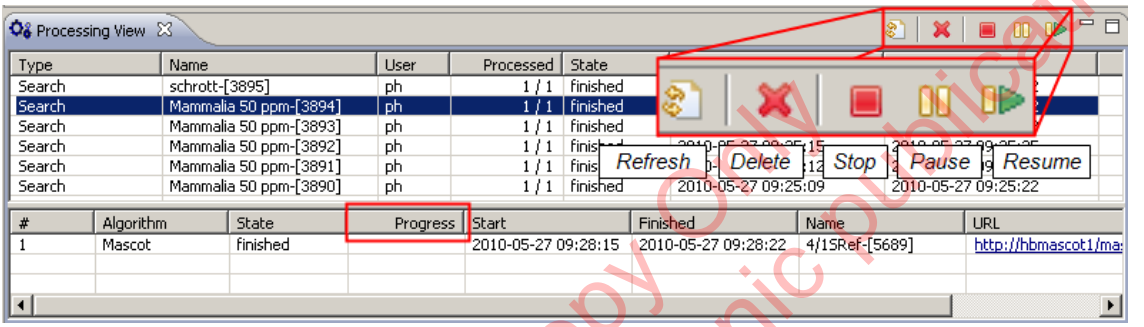

Figure 6-30 Processing View

The Processing View displays the progress of several processes, for example,:

- Data import
- Database searches
- Protein list compilation

The upper part of the view displays a list of processes. Clicking a process in the upper part displays additional information relating to the selected process in the lower part of the view.

During a database search, the **Progress** column displays the progress of each individual step, for example, the search itself, the result parsing, and the result import.

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Table 6-12 Processing View toolbar buttons

Button	Name	ToolTip	Action
	Refresh	Refresh Status (F5)	Update the page content by reloading data from the database.
	Delete	Delete selected Batch(es) (Delete)	Delete the selected batch(es) from the **Processing View** table.
	Stop	Stop selected Batch(es)	Stop processing the selected batch(es).
	Pause	Pause selected Batch(es)	Pause processing the selected batch(es).
	Resume	Resume selected Batch(es)	Resume processing the selected batch(es).

6.4.6.1 Processing View Toolbar and Shortcut Menu

The toolbar in the upper-right corner of the Processing View can be used to control an active process or manage the list of processes. The toolbar commands can be applied simultaneously to multiple processes.

Toolbar commands and the following additional functions can also be accessed through the shortcut menu (right-click in the upper area of the Processing View).

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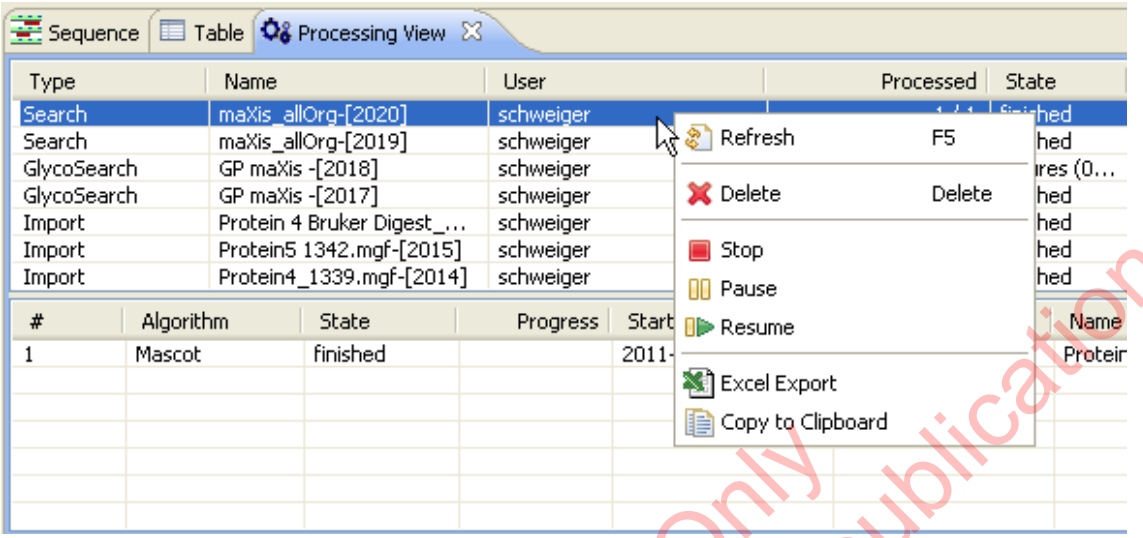

Figure 6-31 Processing View shortcut menu

Excel Export — Automatically exports the current contents of the selected table to an Excel sheet.

Copy to Clipboard — Enables the contents of the selected table to be pasted into spreadsheet applications.

Depending on the **Type** of process, further functions are available in the menu of the lower part of the Processing View.

Right-click the lower table entry and select the relevant option to **Navigate to Search Result** (**Type** = **ProteinExtractor** or **Search**) or **Navigate to Spectrum** (Type = **Import**).

6.4.7 Progress View

The Progress View is opened through the main menu (**Window > Show View > Progress View**).

The Progress View displays the progress of ProteinScape client background processes, such as queries, loading raw LC-MS Survey data or uploading files for MS data import.

Processes can be canceled here by selecting them and clicking **Stop** ( ).

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Table 6-13 Progress View toolbar buttons

Button	Name	ToolTip	Action
	Remove	Remove all completed operations	Delete all finished operations from the **Progress View** table.

6.5 Generating a Scheduled Precursor List (Exclusion SPL)

Each MS/MS Search Result can be used to generate a Scheduled Precursor List (SPL), which contains an entry for each identified peptide. This list can be used as an "Exclusion SPL" for a subsequent LC-MS/MS run. During the run, the instrument will focus fragmentation on unidentified signals and exclude signals that have already been identified.

►► To generate a scheduled precursor list

1. Right-click a Search Result in the **Project Navigator** and select **Generate Exclusion SPL**.

   The **Create Exclusion SPL** dialog opens.

2. Set the desired ***m/z* tolerance** and **RT tolerance** values.
3. Click **OK** to open the **Exclusion SPL Content View**, where the SPL can be reviewed, edited and exported.

   a. Right-click in the **Row** column and select **Remove** to delete an SPL entry.
   b. Right-click in the **Row** column and select **Excel Export** to open the table in Microsoft Excel.
   c. Right-click in the **Row** column and select **Copy to Clipboard** to copy the contents of the table to the clipboard.

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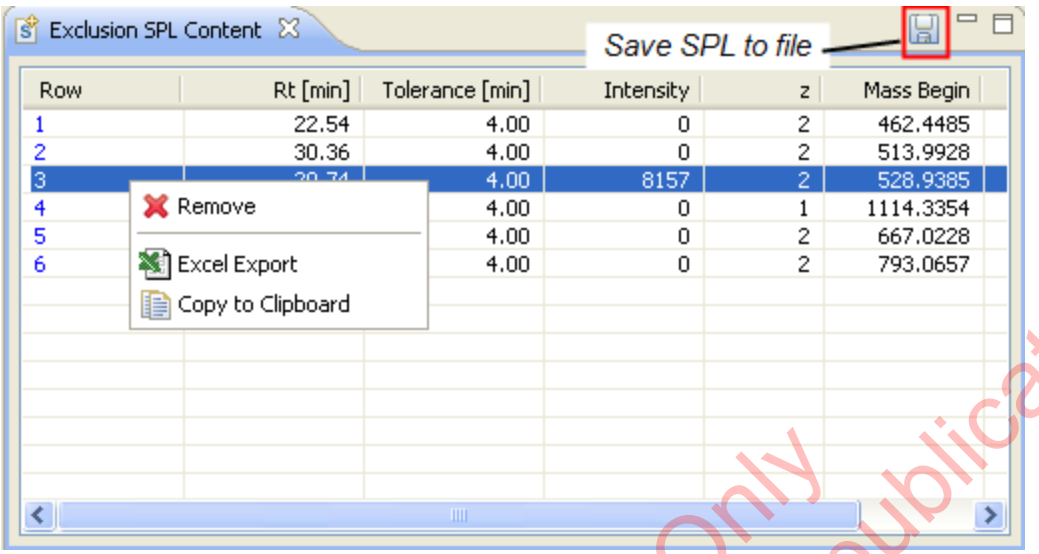

Figure 6-32 Exclusion SPL Content View

4. To save the SPL to a method, click on the diskette symbol in the View's top right corner.

   Note SPLs cannot be saved in the ProteinScape database.

6.6 Robotics

The Proteineer SP spot picking robot under spControl 3.1 can export images and spot lists to ProteinScape. For details, refer to the *spControl User Manual*.

6.7 AutoXecute

AutoXecute is used to automatically transfer single MALDI spectra and LC-MALDI runs to ProteinScape (see section 4.1.3).

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7 Protein Quantitation

ProteinScape supports a number of protein quantitation workflows; including various isobaric, non- isobaric, chemical labeling (SILE), and metabolic labeling (SILAC) workflows and multiplex quantitation experiments.

ProteinScape also supports label-free (LF) quantitation workflows, in which data sets from a high-quality LC-MS system are quantified against each other, without using any internal quantitation standards. Note that LF quantitation workflows are only possible in combination with ProfileAnalysis. Activation of the LF quantitation features in ProteinScape requires download of the LF Quantitation plug-ins from the ProteinScape server (see section 1).

7.1 Quantitation Workflows Using Labeled Samples

In labeled- sample workflows, one of a number of biological samples (cell populations, proteins, peptides) is labeled using a metabolic (Stable Isotope Labeling with Amino acids in Cell culture; SILAC) or chemical (SILE) modification. After mixing and digesting the samples, the resulting peptides can be assigned to the respective labeled or non-labeled sample.

Pairs (or *n*-tuplets) of peaks can be found for each labeled/non-labeled peptide pair and intensity ratios can be calculated.

The available quantitation workflows using stable-isotope labels are:

- **Isobaric SILE** (for example, iTRAQ) — The labeled peptides have the same mass but generate different reporter ions in the MS/MS spectrum. Here, ratio calculation takes place at the MS/MS level where one MS/MS spectrum is generated per *n*-tuplet.
- **Non-isobaric SILE** (for example, ICPL) — Pairs are found at the MS level. For quantitation in ProteinScape, one or more MS/MS spectra are acquired for a pair (or *n*-tuplet).
- **Non-isobaric SILAC** (for example, 13C6 R vs. 12C6 R) — The workflow is the same as for non-isobaric SILE. Labels are introduced by adding isotopically labeled amino acids to cultivation media containing labeled amino acids and not by post-

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lysis chemistry. Typically, two or more isotopic labels (for example,, 13C and 15N) are combined in each labeled amino acid.

Labeled quantitation experiments are indicated in the **Project Navigator** by the Mixed Sample node ( ) . A mixed sample consisting of two or more labeled Samples ( ).

7.1.1 Generating Protein Ratio Data

The ratio of the labeled to the unlabeled sample of a given protein is calculated on the basis of the ratios of its constituent peptides in the two samples.

The following figure shows an overview of a search result with quantitative information. The Protein table columns **Median(L/H)**, **#(L/H)**, **CV[%](L/H)** contain the quantitation information for each protein. The suffix **(L/H)** indicates that the data displays the ratio of the light (**L**) and heavy (**H**) ICPL labels. Sets of columns exist for each ratio definition in multiplex experiments.

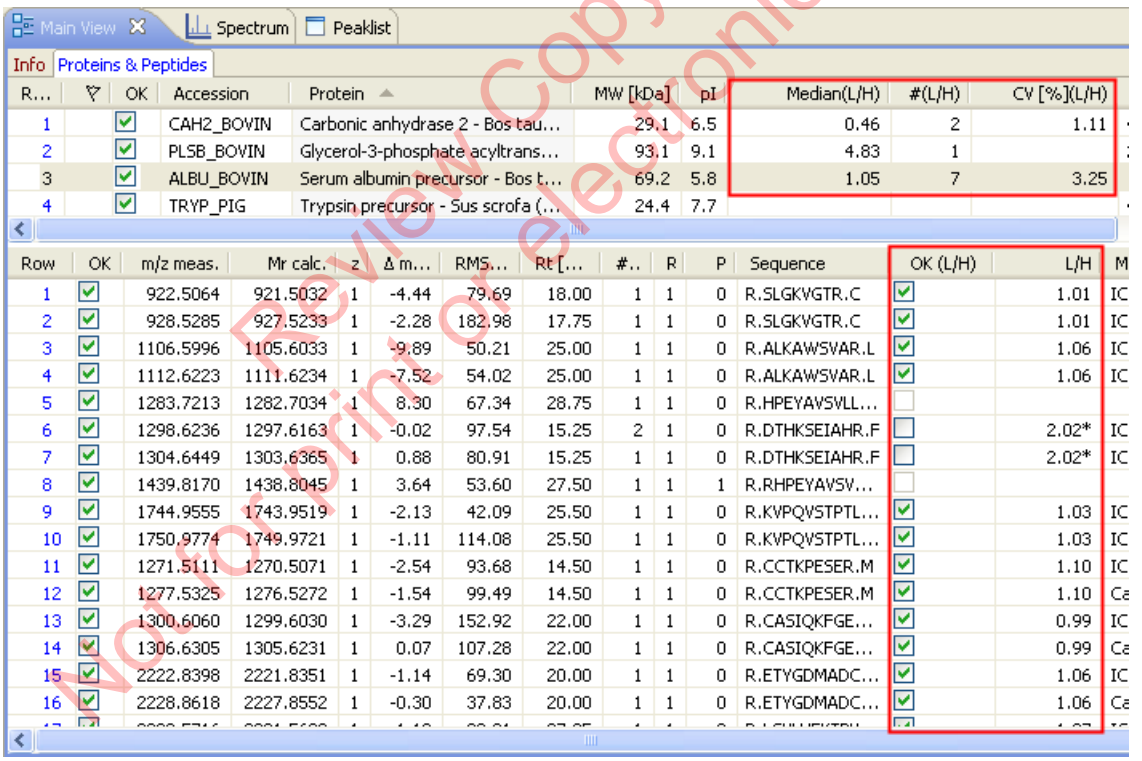

Figure 7-1 Quantitation information displayed in the Protein and Peptide tables

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The Protein table **Median** value is calculated from the peptide pairs, multiplets or buckets identified and is based on a log-normal distribution.

For ratio calculations of each protein, only peptides whose **OK (L/H)** check box is selected in the Peptide table are taken into account.

Outliers are detected automatically. An outlier that has been accepted (**OK** column selected) on the basis of its peptide score is marked with an asterisk.

Extreme values (that is, outside the dynamic range of quantitation) are marked with an up or down arrow in the column displaying the peptide ratios. Neither extreme values nor outliers are included in the protein ratio calculation. An exclamation mark next to the **Median** ratio value of the selected protein in the Protein table indicates the presence of an extreme value in the ratios of its constituent peptides.

7.1.1.1 Changing the Peptides Used for Calculating Protein Ratios

Clicking a check box in the **OK (L/H)** column switches its status between selected and cleared. To change multiple check boxes, select and right-click the desired Peptide table entries and select **Quantitation Selection** from the shortcut menu. Select or clear the respective ratio check box in the **Quantitation Selection** dialog and click **OK**.

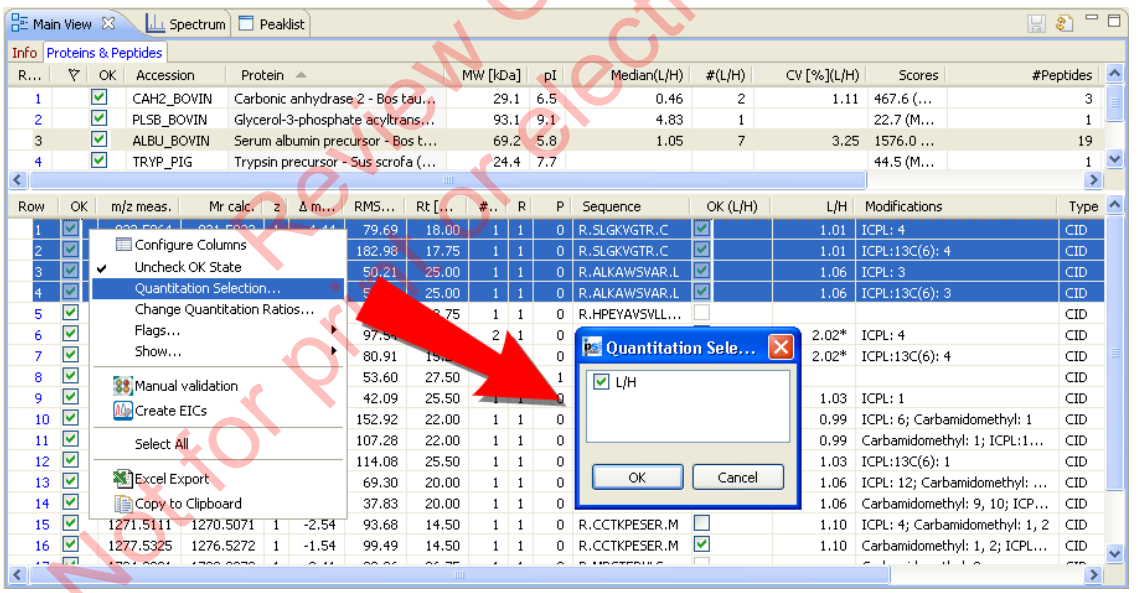

Figure 7-2 Changing the status of multiple OK(L/H) check boxes using the Quantitation Selection dialog

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If the check marks in the Peptide table **OK (L/H)** column are changed manually, the new protein table values (sequence coverage, number of peptides identified, number of peptides quantified, median, CV) are recalculated automatically and the **Quantitation Statistics** View is updated. This may take some time if the communication between the server and client is slow and/or many check marks are altered. If this is the case, automatic updating can be switched off by clicking the active **Auto Refresh** button ( ) in the main toolbar.

7.1.1.2 Changing the Peptide Ratio Values Used for Calculating Protein Ratios

The ratio value of individual peptides may be changed manually. Highlight and right-click the desired peptide entry and select **Change Quantitation Ratios** in the shortcut menu. A new ratio value can then be typed into the **Change Quantitation Ratios** dialog. The original value is overwritten and can only be restored by repeating the quantitation on the original Search Result.

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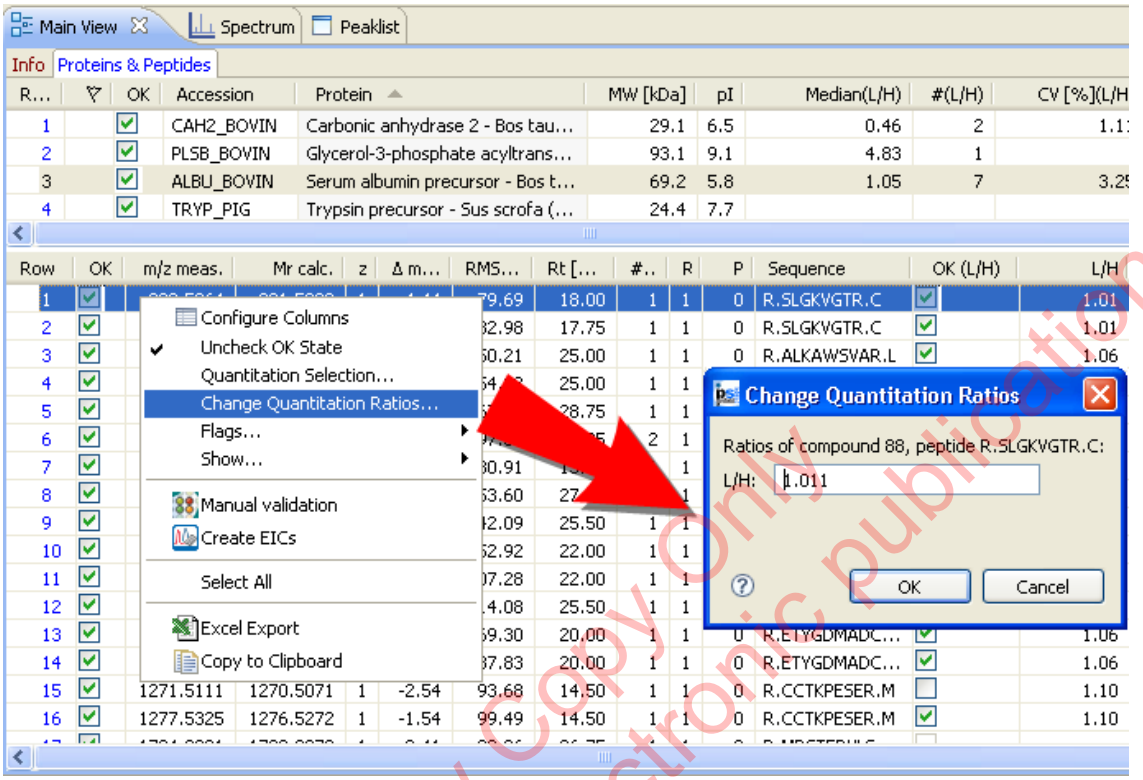

Figure 7-3 **Changing the quantitation ratio of a peptide using the Change Quantitation Ratios dialog**

7.1.2 Displaying Protein and Peptide Ratio Data

The Protein table in the **Proteins & Peptides** tab of the Search Result Main View displays the **Median** calculated ratio of labeled to non-labeled proteins, together with some statistical parameters (for example, CV[%]).

The Peptide table in the **Proteins & Peptides** tab of the Search Result Main View displays the ratios of heavy to light peptides in the column **L/H**.

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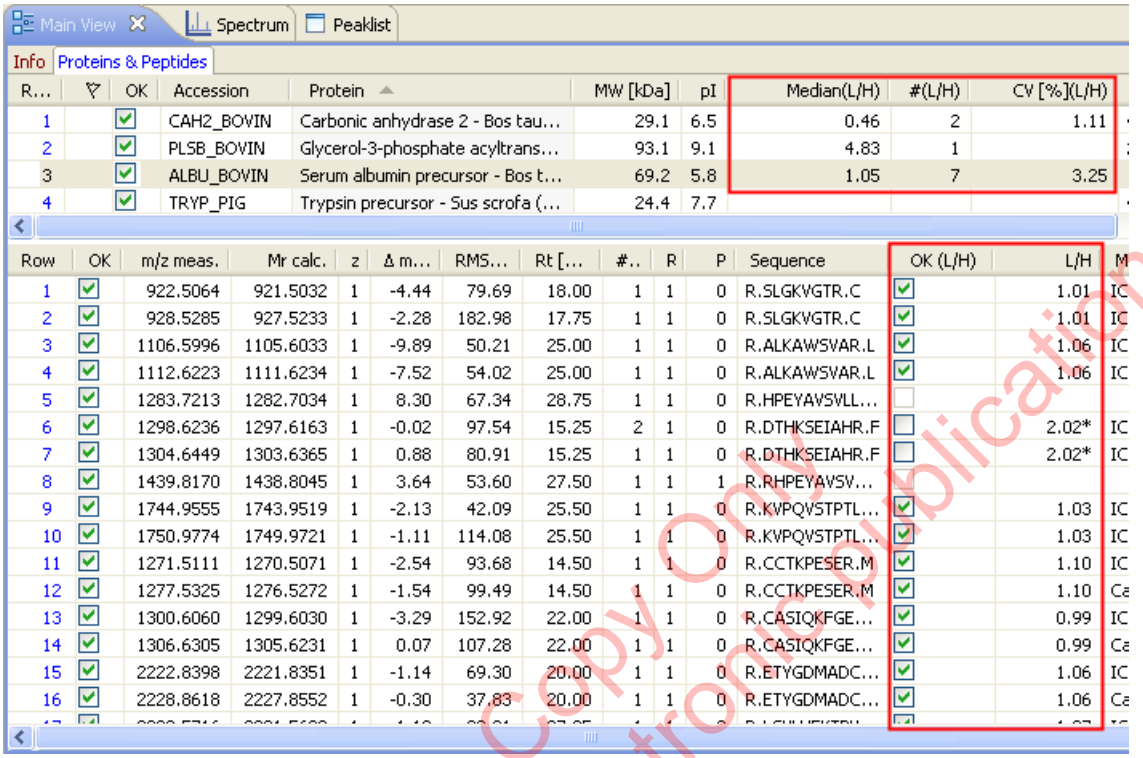

Figure 7-4 Quantitation ratios displayed in the L/H column of the peptide table

The box and whisker plot in the **Quantitation Statistics** View (see section 7.1.4) summarizes the statistical analysis of the accepted peptides.

7.1.3 Normalization

The main purpose of quantitation experiments is usually the identification of proteins that appear to be up- or down-regulated amongst a (vast) majority of unregulated proteins. The peptide ratios of unregulated proteins should show a normal distribution about the median value (= 1). However, errors in generation and preparation of samples may lead to a shift of the median. The resulting quantitation errors can be corrected by a normalization step.

ProteinScape offers a simple normalization feature in which individual peptide ratios are divided by a normalization factor. This value can be the overall median of all peptides or a user-defined denominator.

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To select a normalization factor, click **Normalization** in the **Search Results Info** section of a Search Result's Main View. Select **By overall median** to use the overall median of all peptides. Alternatively, clear the **By overall median** check box and type a denominator in the **Factor** field next to the relevant ratio. Clicking **OK** will apply the normalization factor to the entire search result.

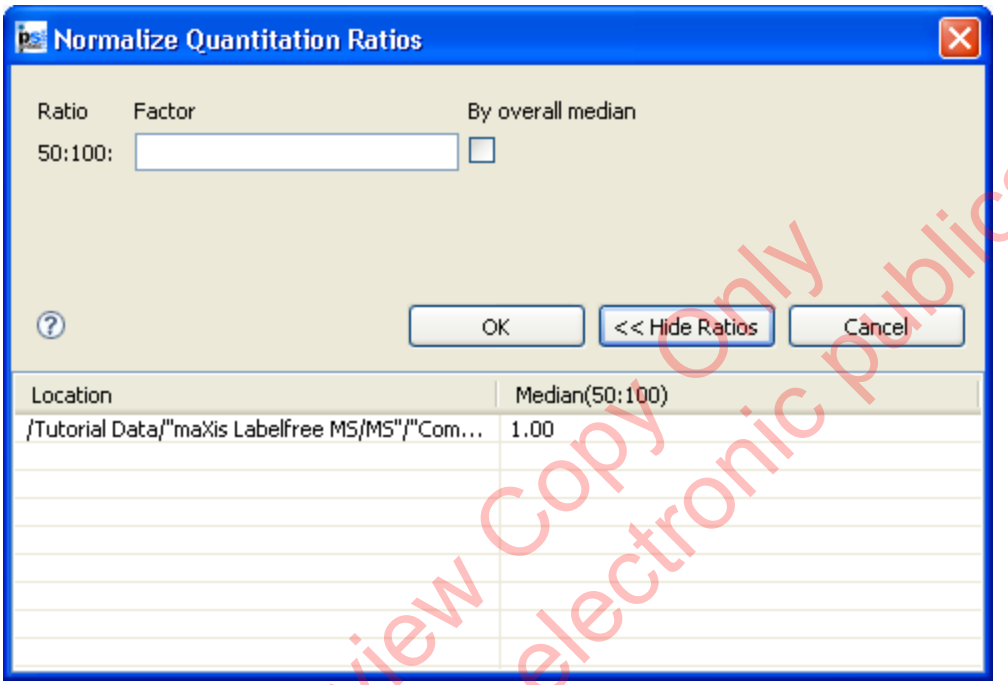

Figure 7-5 Normalize Quantitation Ratios dialog

7.1.4 Quantitation Statistics View

The Quantitation Statistics View is opened through the main menu (**Window > Show View > Quantitation Statistics**) and contains three pages; **Ratio / Score**, **Ratio / Intensity** and **Peptide Statistics**.

Ratio / Score page

The **Ratio / Score** page displays quantitation ratio (for instance heavy/light) plots of the protein selected in the protein table (top panel) and its peptides (lower panel).

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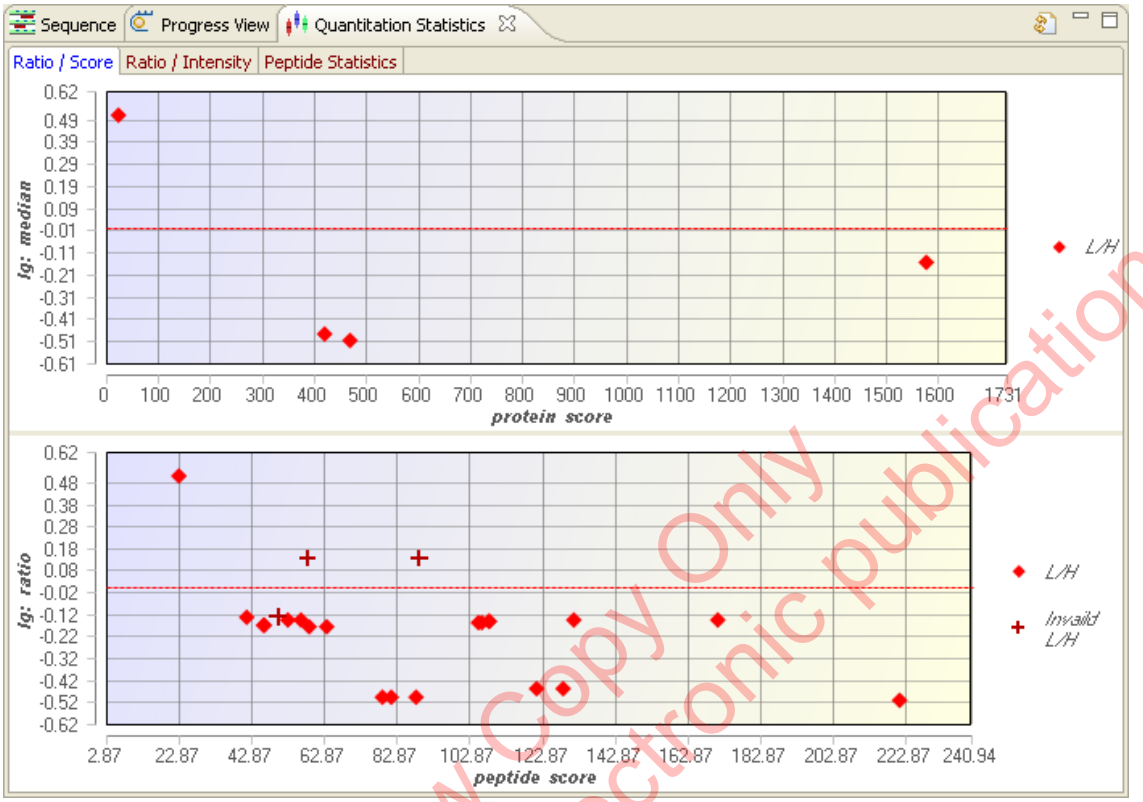

Figure 7-6 Quantitation Statistics Ratio / Score page

The upper protein panel displays a plot of the median quantitation ratio for all peptides in each protein against the protein score. This plot provides a fast way to check the confidence of protein identification and the level of up/down-regulation.

The lower peptide panel displays a plot of the quantitation ratio for each peptide in the selected protein against the peptide score and gives an indication of the quality of the quantitation data.

Peptides that have a check mark in the **Quant OK** column of the Peptide table and are used for protein quantitation are indicated by diamonds. Peptides that have no check mark in the **Quant OK** column (outliers or peptides with a very low Mascot score) are not used for protein quantitation are indicated by crosses.

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Ratio / Intensity page

The **Ratio / Intensity** page displays a plot of the quantitation ratio for each peptide in the selected protein against the peptide intensity and gives an indication of the quality of the quantitation data.

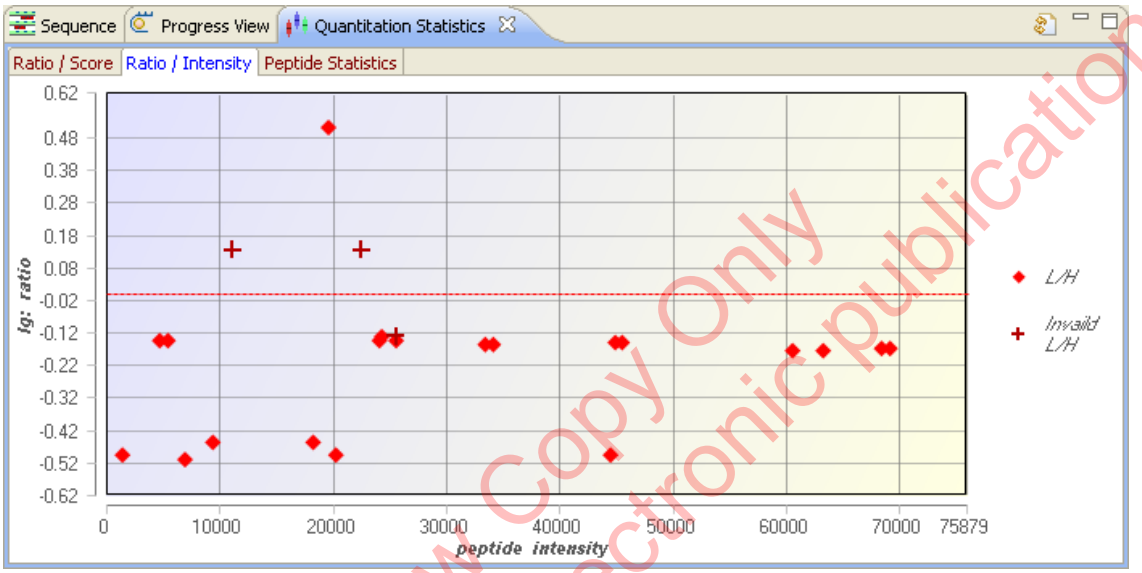

Figure 7-7 Quantitation Statistics Ratio / Intensity page

Peptide Statistics

The **Peptide Statistics** page displays a box and whisker plot of quantitation data from labeled samples. The height of the red rectangle is equivalent to the difference between the upper (**Q3**) and lower (**Q1**) quartiles, the black line indicates the median value (**Median**). The black dot indicates the arithmetic mean (**Mean**). The highest (**Max.**) and lowest (**Min.**) value are marked by the â€œwhiskersâ€ . Moving the mouse pointer over the rectangle opens a ToolTip that displays the respective values.

The **Peptide Statistics** page displays a box and whisker plot of quantitation data from labeled samples. The height of the red rectangle is equivalent to the difference between the upper (**Q3**) and lower (**Q1**) quartiles, the black line indicates the median value (**Median**). The black dot indicates the arithmetic mean (**Mean**). The highest (**Max.**) and lowest (**Min.**) value are marked by the â€œwhiskersâ€ . Moving the mouse pointer over the rectangle opens a ToolTip that displays the respective values.

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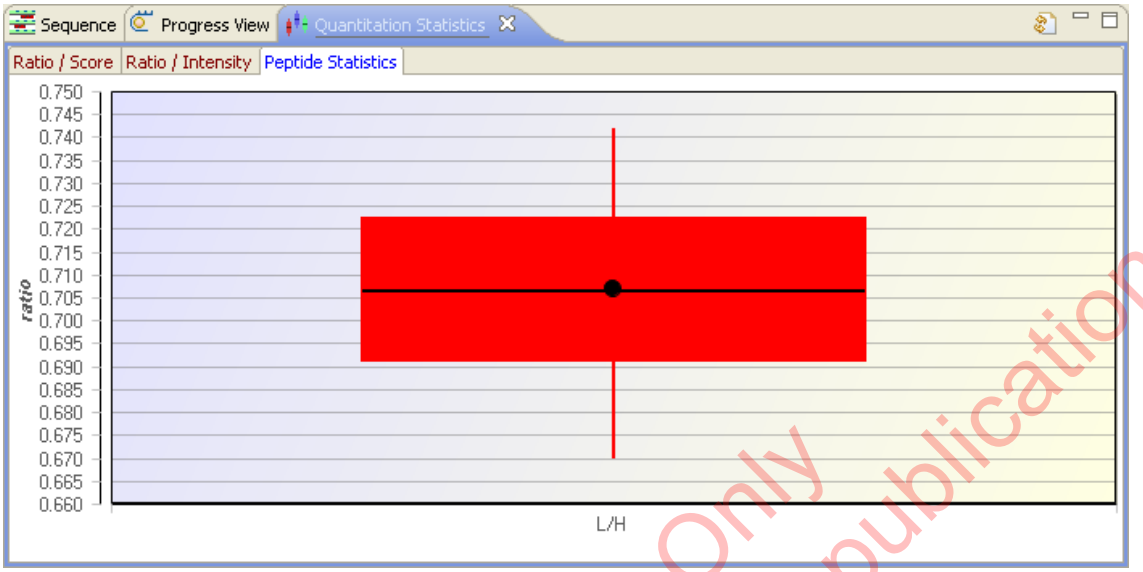

Figure 7-8 Quantitation Statistics Peptide Statistics page

Quantitation Statistics View Shortcut Menu

The Quantitation Statistics View shortcut menu is launched by right-clicking anywhere within the View.

Show Original Size — Reset view after zooming action.

Configure Series — Show/Hide data series

Show all peptides — Display all peptides

Copy to Clipboard — Copy image to clipboard

Save As — Save image as graphic file

Scale y axis to lg — Select to change y-axis from arithmetic to logarithmic scale

Moving the mouse pointer over a point in a plot displays the name of the protein/peptide and quantitation data in a ToolTip, enabling fast identification of outliers.

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7.1.5 Quantitative Labeling Experiments using LC-ESI Data

Quantitative workflows using LC-ESI data require communication between ProteinScape and WARP-LC.

When the search is finished, the quantitation can be started by clicking the **SILE Quantitation** button on the **Info** tab of the Search Result Main View or selecting **SILE Quantitation** from the Search Result shortcut menu (see Figure 7-9).

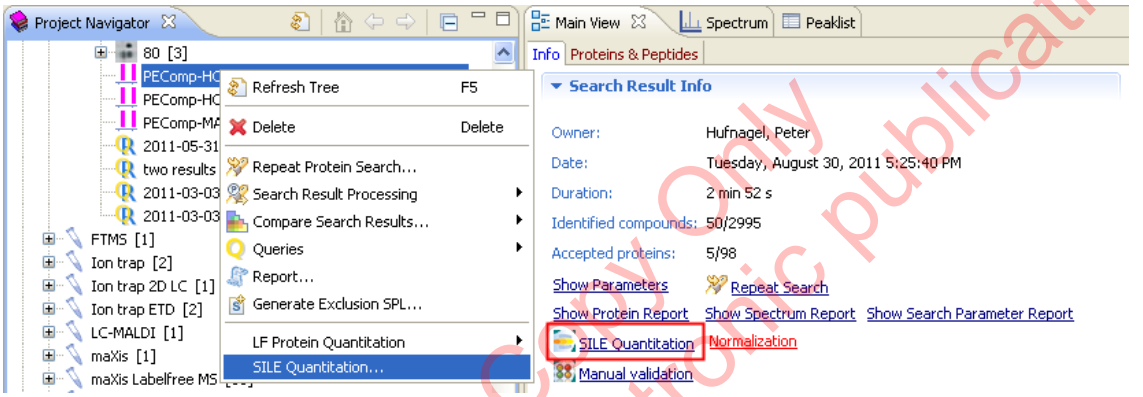

Figure 7-9 Starting a SILE Quantitation

Clicking SILE Quantitation starts WARP-LC and requests user input. An appropriate WARP-LC workflow type that contains all parameters for the quantitation must be selected on the **Workflow** page of the WARP-LC Method Editor (see Figure 7-10).

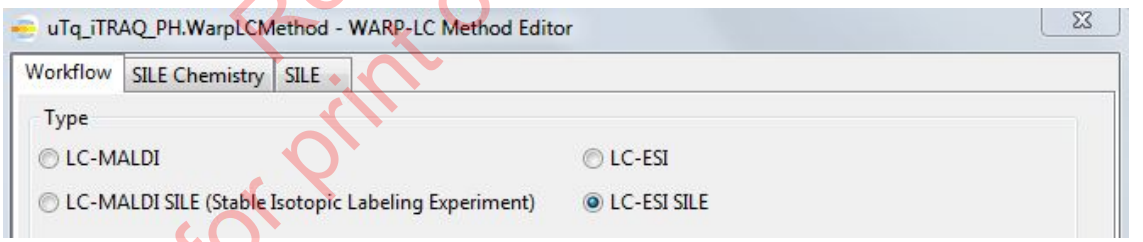

Figure 7-10 Workflow page of the WARP-LC Method Editor

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SILE Chemistry

The **SILE Chemistry** page of the WARP-LC Method Editor contains the chemical definitions of the SILE labels (see Figure 7-11). Before assigning labels to Biological States, a ProteinScape protein search method must be specified on the**MALDI MS/MS** page (see Figure 7-15). The label definitions are read out from the ProteinScape search method.

Biological State refers to the individual labeled samples (for example, treated vs. untreated). Each **Biological State** can have one or more chemical or metabolic label (**Labels**). The **Biological States** can be combined in the section **Ratio Definitions** for the calculation of the respective intensity ratios.

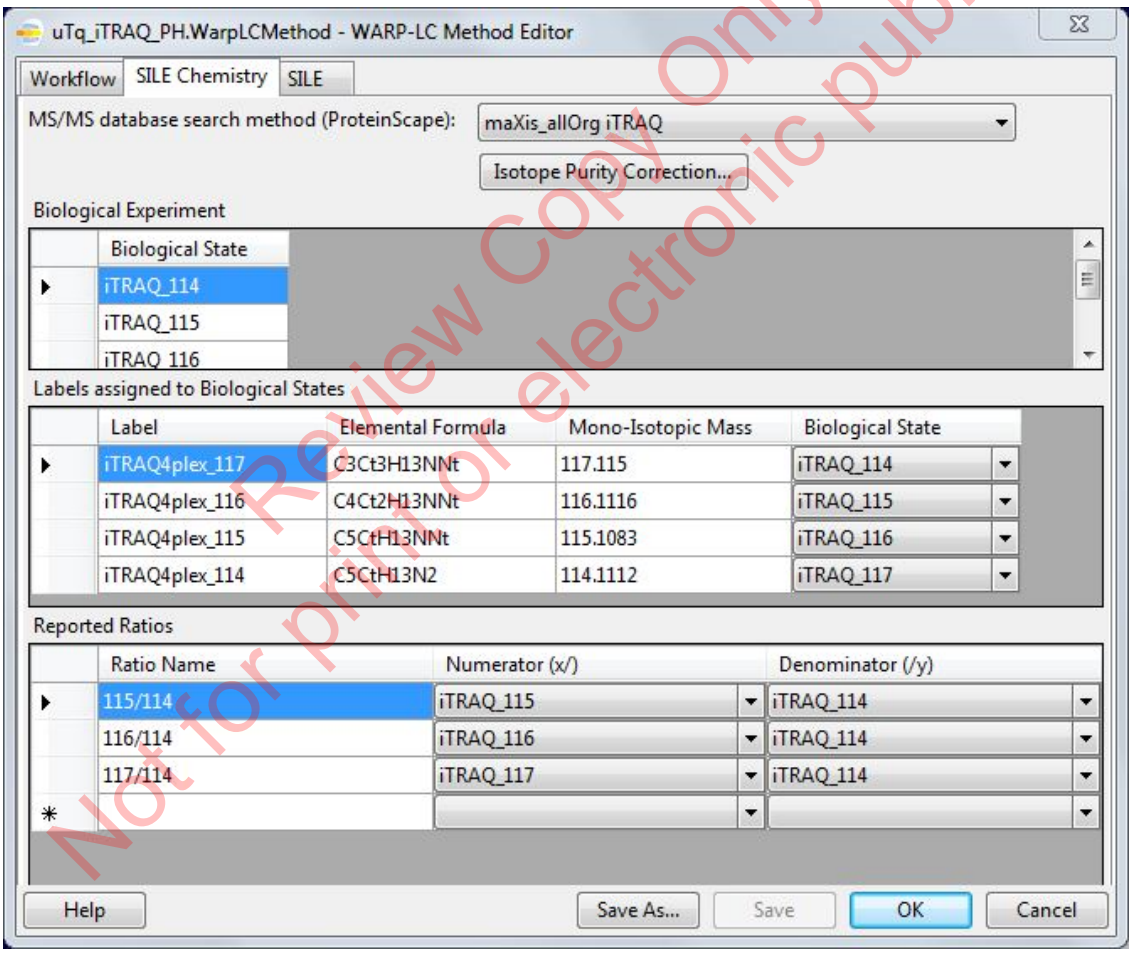

Figure 7-11 Parameters on the SILE Chemistry page of the WARP-LC Method Editor

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The **SILE** page of the WARP-LC Method Editor contains parameters for the detection of SILE pairs / *n*-tuplets (see 7.1.5).

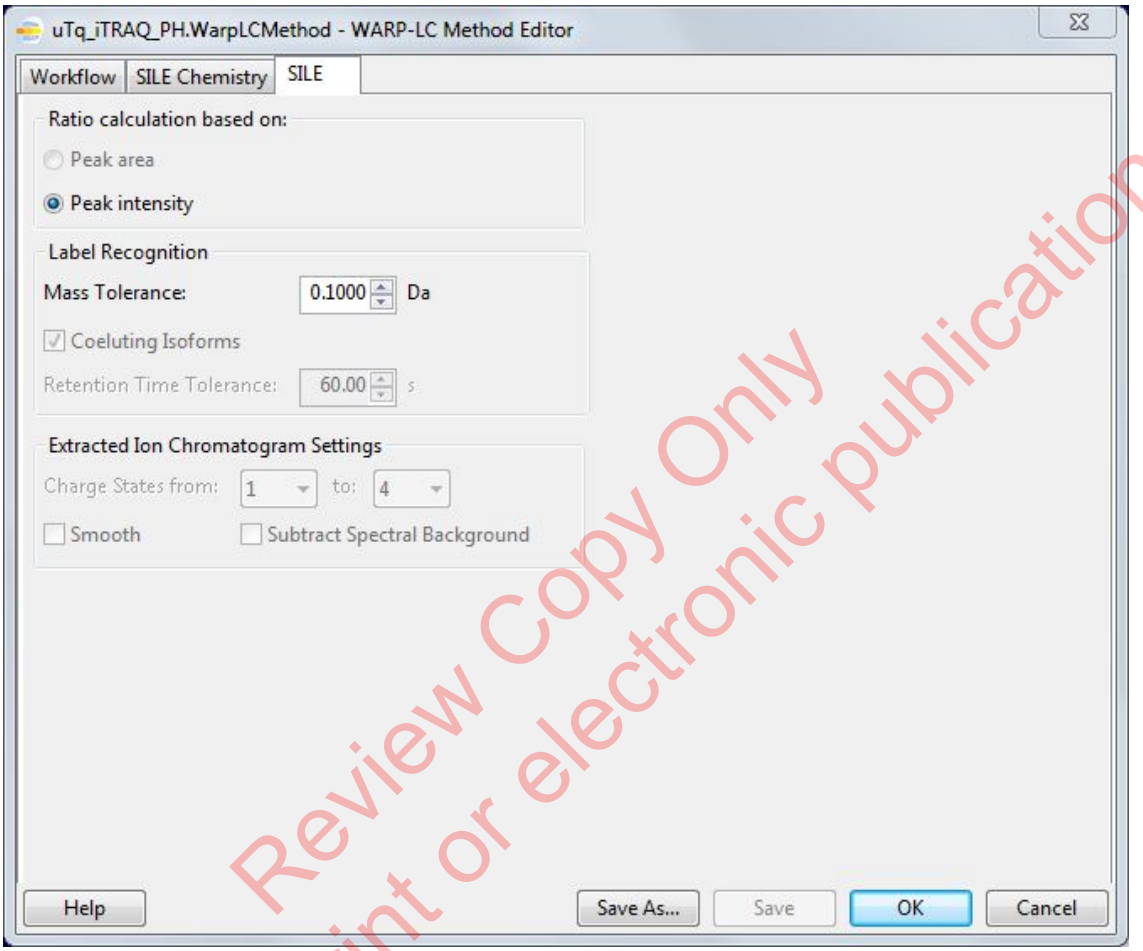

Figure 7-12 Parameters on the SILE page of the WARP-LC Method Editor

Generation of EICs for non-isobaricSILE quantitations

For non-isobaric quantitation, the best precision is obtained if the areas of the chromatographic peaks are determined. The integration of the MS intensities over a chromatographic peak in an Extracted Ion Chromatogram (EIC) is time-consuming procedure. Therefore, this step is only performed for SILE pairs with at least one identified peptide.

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WARP-LC triggers DataAnalysis to generate the required EICs and calculate the integrated intensities. WARP-LC calculates the SILE ratios for all pairs and sends the results back to ProteinScape where the protein ratios are calculated (see section 7.1.2).

7.1.6 Quantitative Labeling Experiments using LC-MALDI Data

Quantitative workflows using LC-MALDI data require intensive communication between ProteinScape and WARP-LC.

7.1.6.1 Data Acquisition in WARP-LC

For general information about LC-MALDI data acquisition, refer to the *WARP-LC User Manual*.

Briefly, an AutoXecute run is either built up from an LC-MALDI fractionation using the Proteineer FC target spotting robot or from scratch using the AutoXecute run editor.

A WARP-LC method that contains parameters for the calculation of **Compounds** and the targeted acquisition of MS/MS spectra is assigned to the whole run (see Figure 7-13).

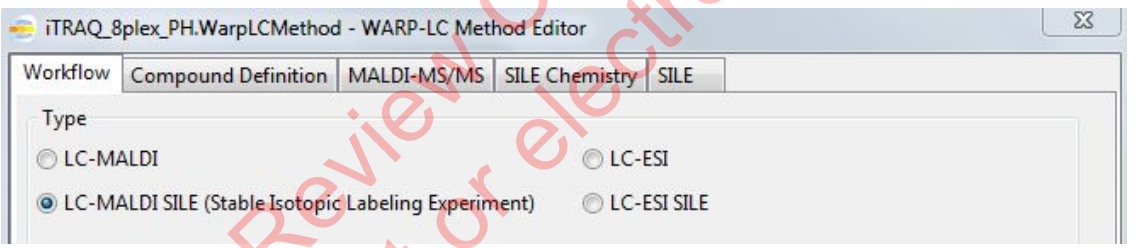

Figure 7-13 Selecting the LC-ESI-MALDI option on the Workflow page of the WARP-LC Method Editor

7.1.6.2 Finding SILE Pairs and Intensity Ratio Calculation in WARP-LC

The WARP-LC method contains parameters for finding SILE pairs as well as definitions of the SILE labels (sites and mass shifts). SILE pairs (*n*-tuplets) are found in the data by examining mass differences between the Compounds' precursor masses.

As for ESI methods, the **SILE Chemistry** page of the WARP-LC MALDI method contains the definitions of the SILE labels (see section 7.1.5). Before assigning labels to Biological States, a ProteinScape protein search method must be specified on the **MALDI MS/MS**

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page (see Figure 7-15). The label definitions are read out from the ProteinScape search method.

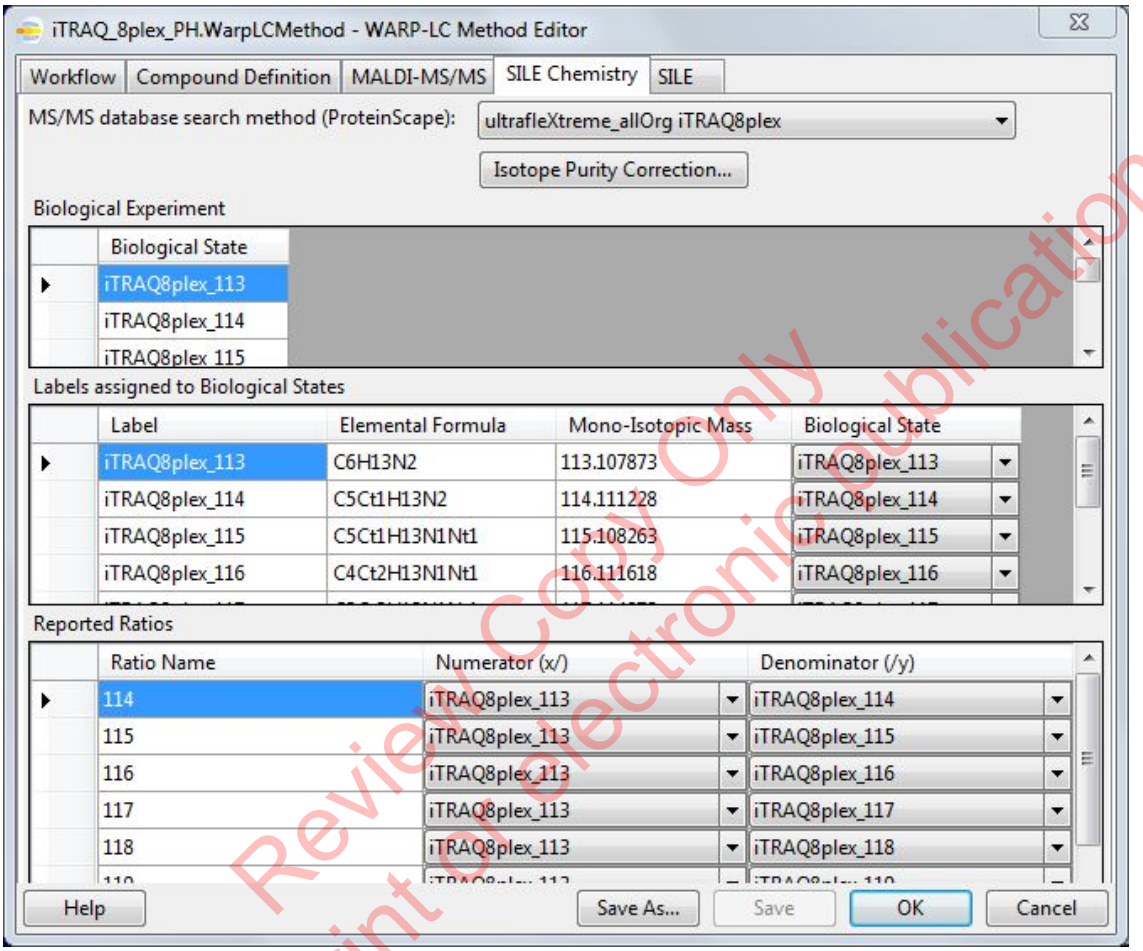

Figure 7-14 Parameters on the SILE Chemistry page of the WARP-LC Method Editor

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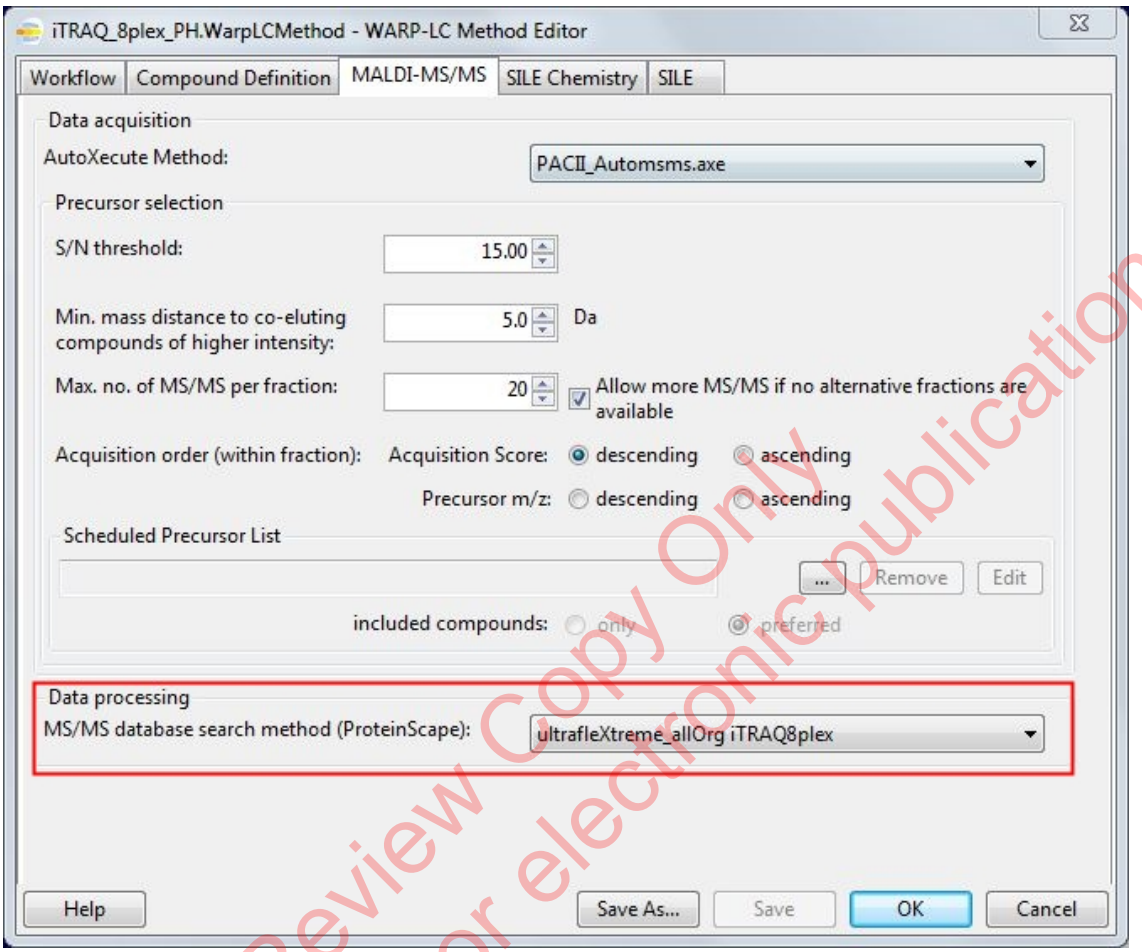

Figure 7-15 Parameters on the MALDI-MS/MS page of the WARP-LC Method Editor

The SILE page of the WARP-LC method contains parameters for the detection of SILE pairs / *n*-tuplets.

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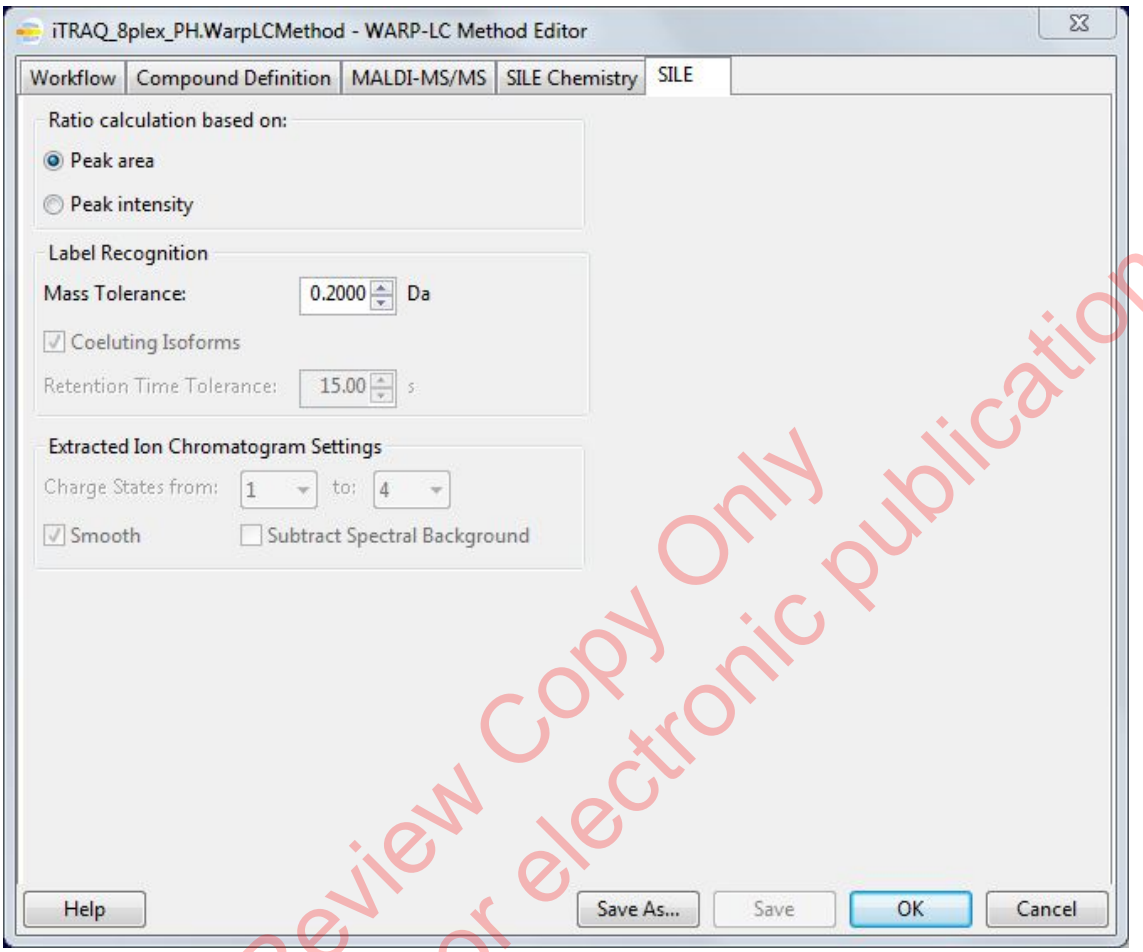

Figure 7-16 Parameters on the SILE page of the WARP-LC Method Editor

7.1.6.3 Linking to a Protein Database Search in ProteinScape

After **Compound** calculation and SILE pair detection, the **Compounds** are exported to ProteinScape. This happens automatically if a ProteinScape search method is selected from the drop-down list in the **Data Processing** area of the **MALDI MS/MS** page of the WARP-LC Method Editor (see Figure 7-15). Note that for ESI workflows, the ProteinScape method is specified on the **Data processing** page (see Figure 7-17).

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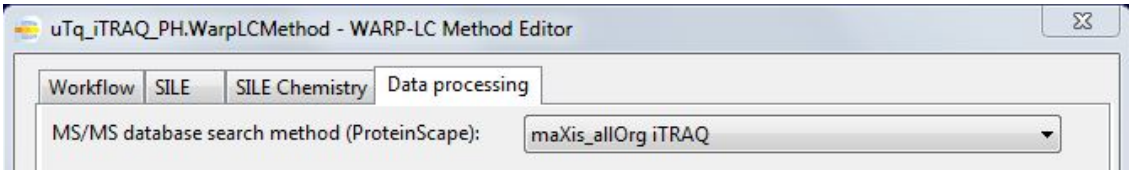

Figure 7-17 ProteinScape search method selected in the WARP-LC Method Editor

The list contains all accessible search methods on the ProteinScape server.

7.1.6.4 Defining Appropriate Modifications in WARP-LC and ProteinScape

The ProteinScape Search Method should include the **Modification**s corresponding to those in the **Labels** area of the **SILE Chemistry** page of the **WARP-LC Method Editor**.

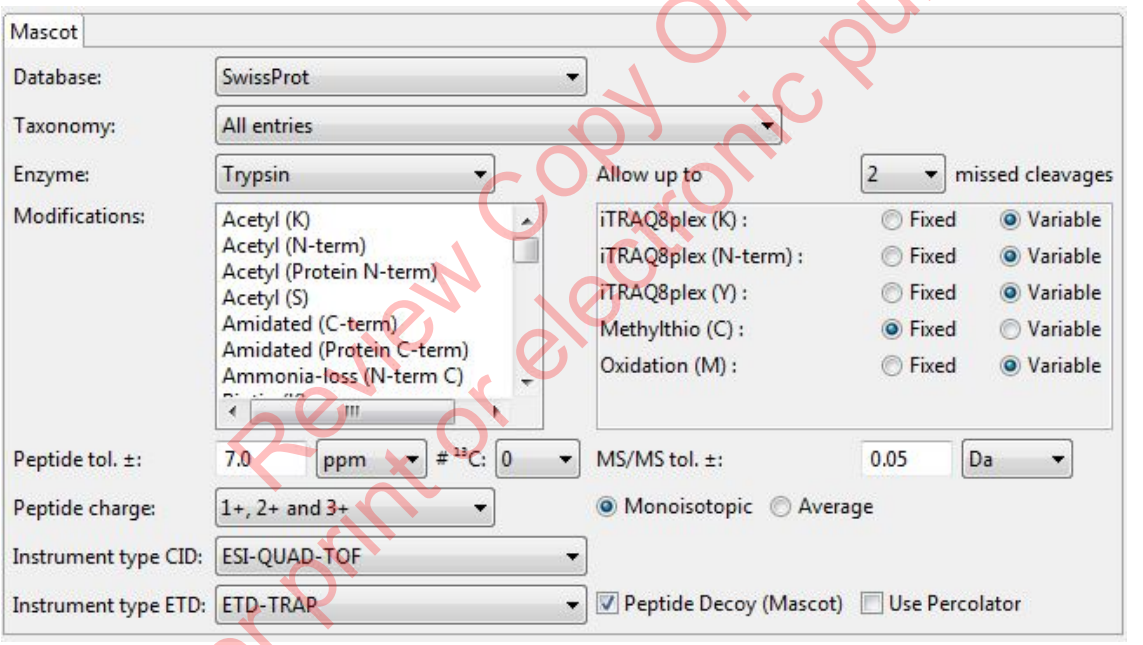

Figure 7-18 Modifications in the ProteinScape Protein Search parameters

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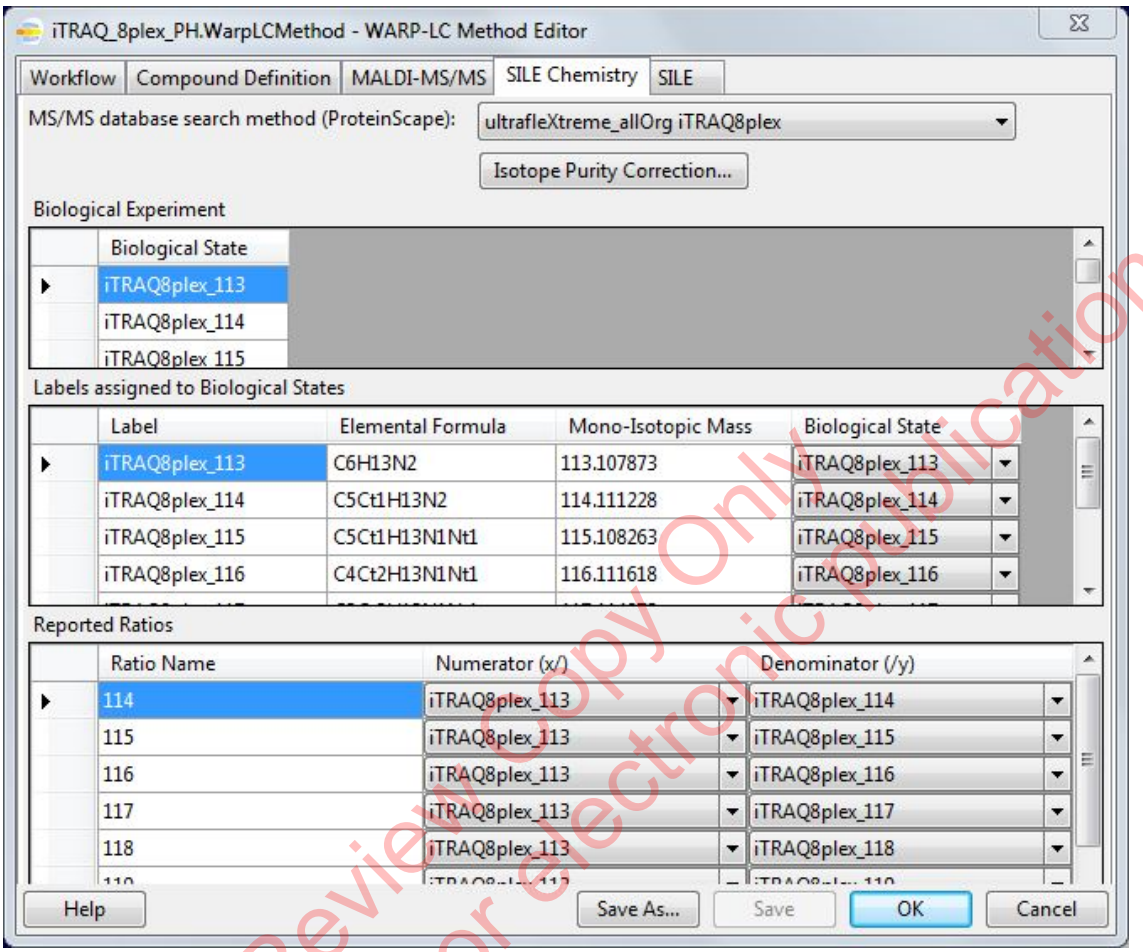

Figure 7-19 Parameters on the SILE Chemistry page of the WARP-LC Method Editor

After the protein search, the Search Result is automatically sent to WARP-LC where the peptide ratios are inserted into the Peptide table and the data is sent back to ProteinScape where a protein ratio is calculated for each protein.

7.1.7 SILE Quantitation in 2D Separation Workflows

ProteinScape enables a SILE Quantitation to be started from ProteinExtractor Search Results that are generated by compiling a protein list from a number of single ProteinExtractor Search Results.

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7.2 Label-Free Quantitation

Quantitation of changes in biological systems that result from certain treatments or perturbations is an important task in proteomics. Label-free (LF) quantitation methods aim to directly compare mass spectrometric signal intensity across multiple liquid chromatography (LC) runs without using an internal standard.

The main advantages of the label-free approach are a simple biochemical workflow and — due to the elimination of labeling steps — reduced sample consumption and costs. It should however be noted that label-free quantitation is strongly influenced by all sample-handling steps, such as separation, concentration or digestion. Accurate quantitation can be obtained by carefully controlling the sample-handling steps and measuring a sufficient number of replicates.

A detailed description of label-free quantitation workflows can be found in the tutorial *Label-free Proteomics Quantification*, which is included in PDF form in the ProteinScape installation.

We highly recommended that the Project structure used for label-free quantitation exactly matches that used in the tutorial (see Figure 7-20).

The Project should contain a Sample node for each abundance ratio. All LC-MS data sets directly contributing to each ratio should be listed directly below their respective Sample node.

The Project should also contain a Sample node containing all LC-MS/MS data sets.

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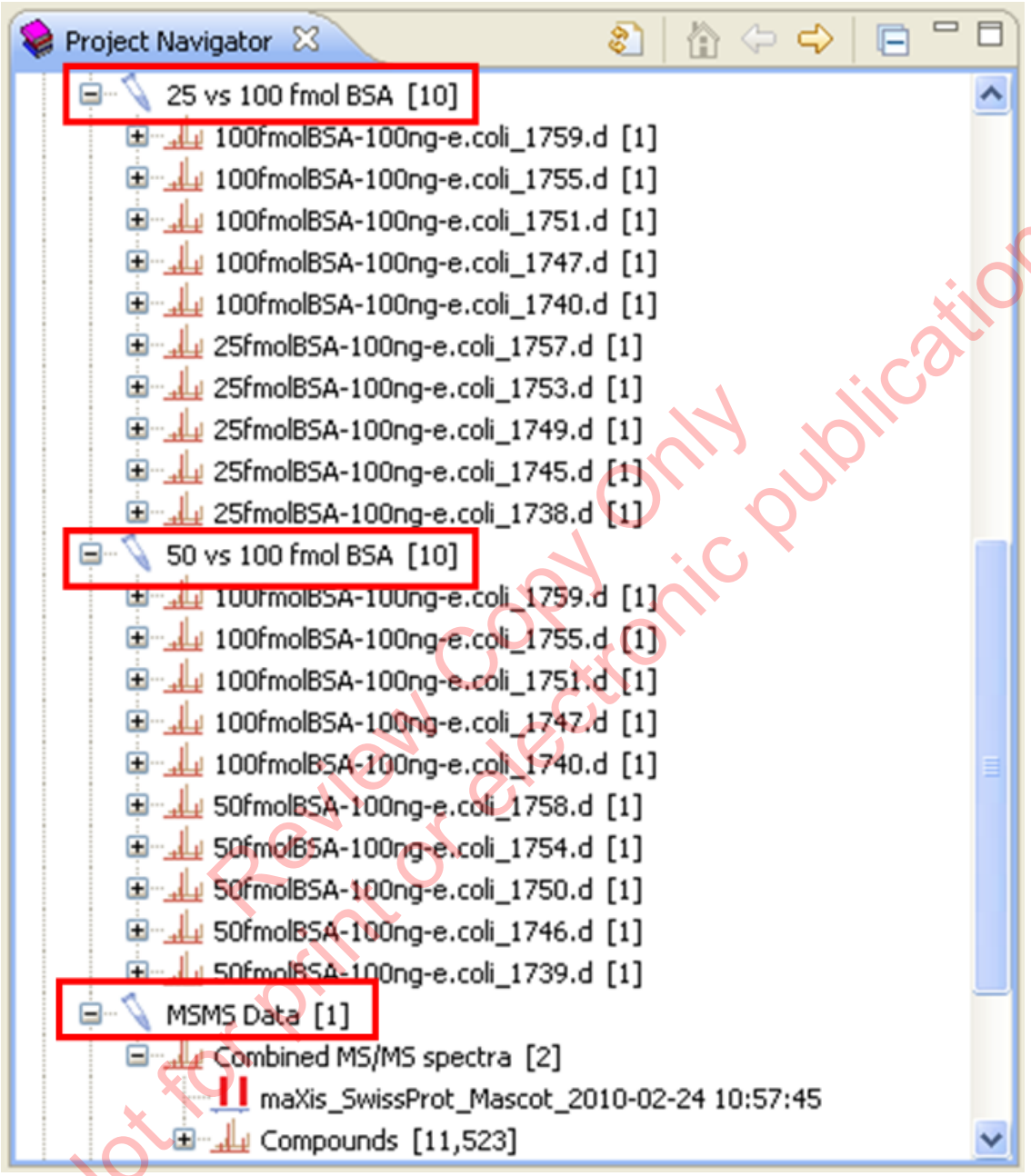

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Figure 7-20 Recommended project structure for label-free quantitation

7.2.1 Data Requirements for Performing Label-Free Quantitation

Label-free quantitation data consist of a number of LC-MS runs for each biological state plus a number of LC-MS/MS runs. Quantitation of two biological states is based on LC-MS runs and performed using ProfileAnalyis software. The LC-MS data and the quantitation results are exported to ProteinScape by ProfileAnalysis. The corresponding LC-MS/MS data sets are imported into ProteinScape using the **Import MS-Data** command in the Project Navigator (see section 4.1.1) or by automatic transfer using the PushDaemon (see section 4.1.2).

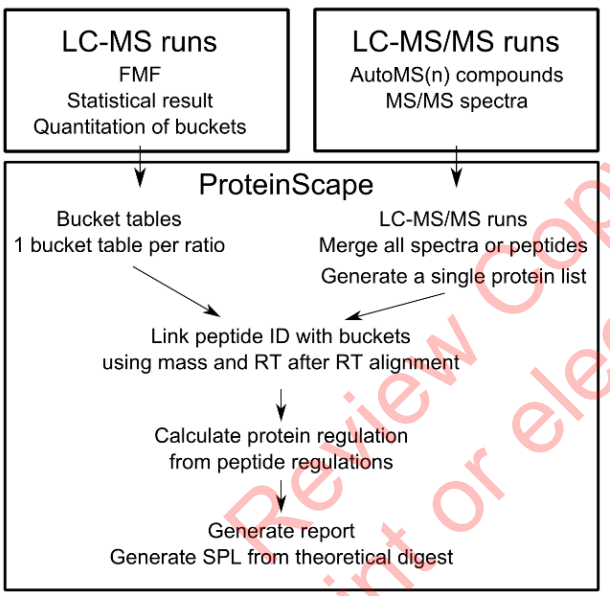

Figure 7-21 Label-free quantitation processing steps in ProteinScape

For LF quantitation, quantitative information (FindMolecularFeatures (FMF) results, t-test, and abundance ratios) are imported into ProteinScape (see Figure 7-21). LC-MS/MS runs are imported and a single protein list is generated from all LC-MS/MS runs in a separate process. When this protein list is available, quantitative information is linked to the identified peptides using mass and retention time as assignment criteria. When quantitation of peptides is available, protein abundance ratios are calculated and a report generated. In addition, Scheduled Precursor Lists (SPLs) can be generated for validations and improvements of identification and quantitation results.

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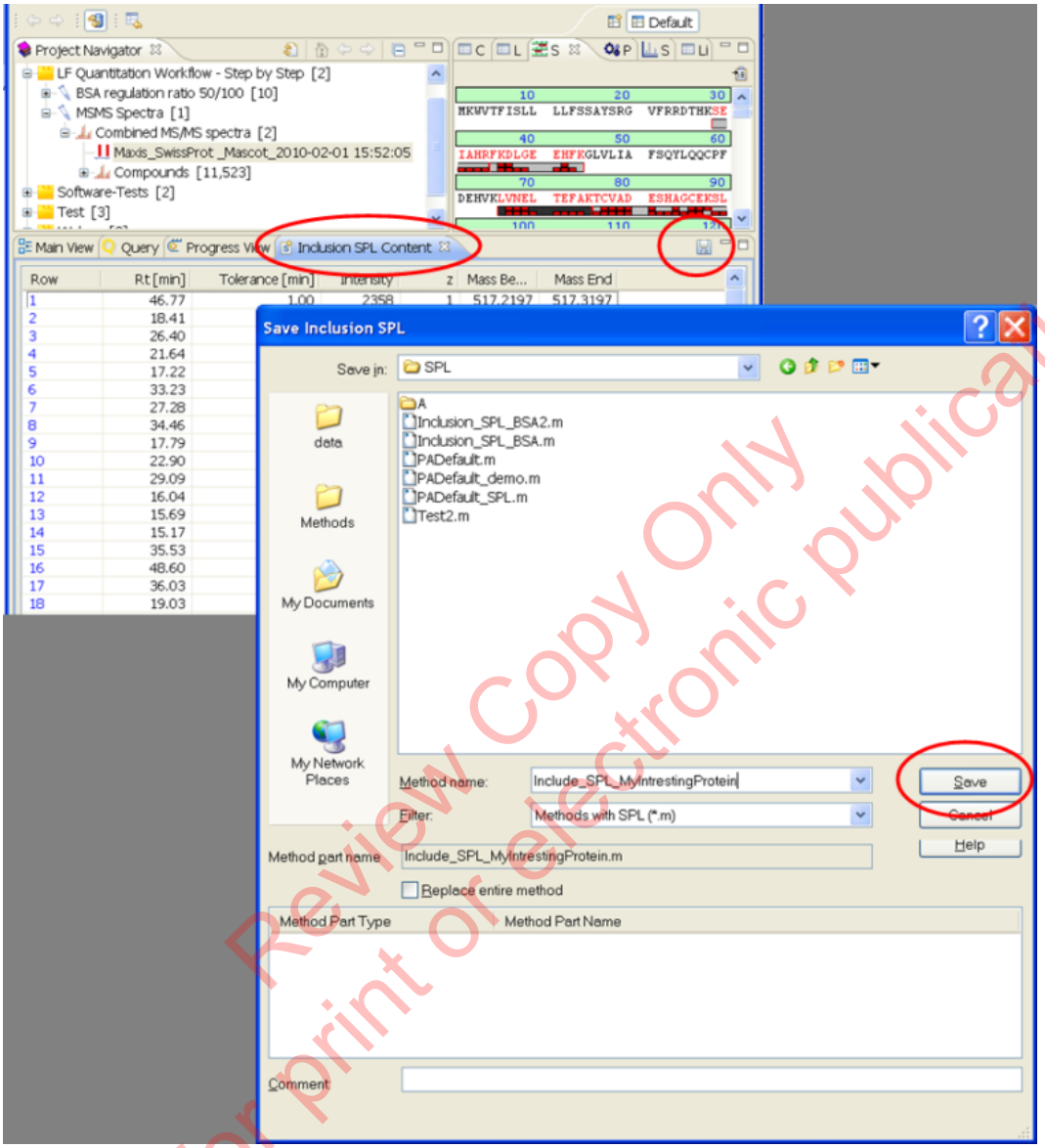

Figure 7-22 Saving an inclusion SPL generated from a theoretical digest

The SPL is saved as method part and should be used in a HyStar Method Set or in the WARP-LC method when performing an LC-MALDI experiment. Refer to the tutorial *Label-free Proteomics Quantification* for further details on how to use an SPL for acquisition.

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7.2.2 Generating a Single Protein List from Multiple LC-MS/MS Runs

If more than one LC-MS/MS dataset has been imported, there are two ways of producing a combined search result in ProteinScape:

- Combining data sets before the protein database search (for example, using Mascot as a search engine).
- Using the ProteinExtractor.

7.2.2.1 Combining Data Sets Before the Protein Database Search

►► To combine data sets before the protein database search

1. In the Project Navigator tree, right-click the Sample containing the LC-MS/MS data sets and select **Combine MS/MS spectra**.

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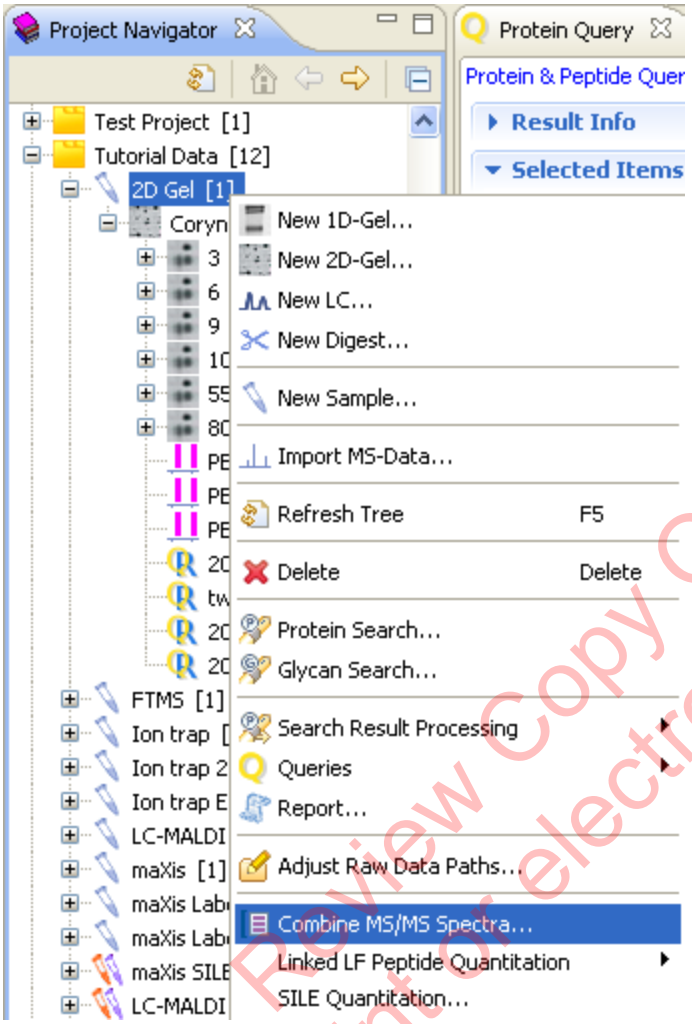

Figure 7-23 Combining MS/MS spectra in a Sample node

2. Select the data sets to be combined in the **Select spectra** field of the **Combine MS/MS spectra** dialog and click **OK**.

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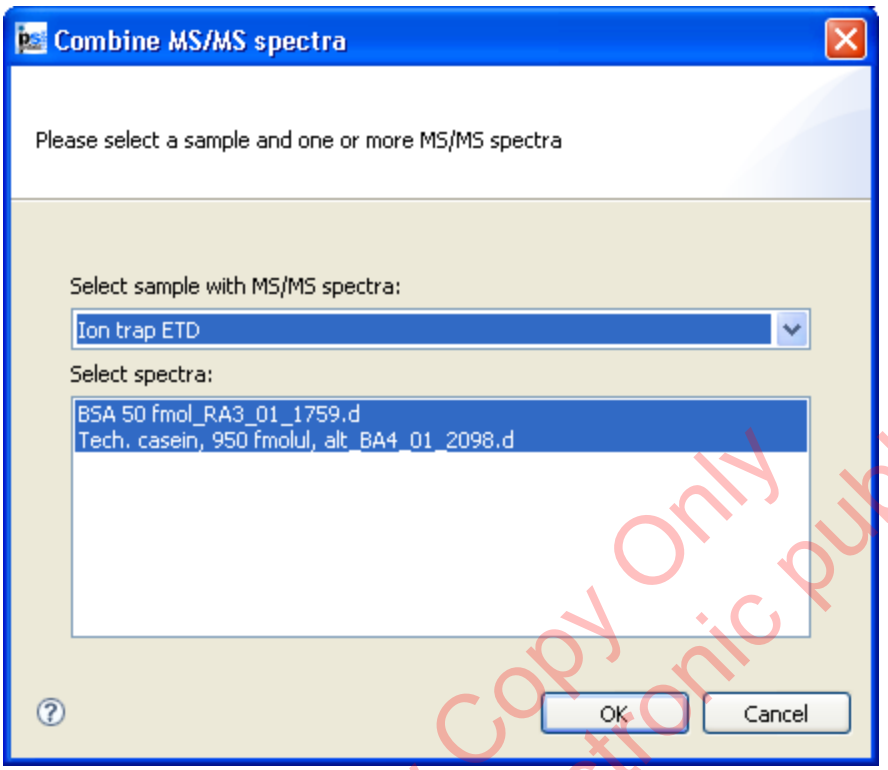

Figure 7-24 Combine MS/MS spectra dialog

The selected data sets will be replaced by a single data set named `Combined MS/MS spectra`.

Note The compound numbers of the original datasets will be retained, meaning that in the combined dataset the compound numbers are no longer unique.

3. After all MS/MS spectra of the LC runs are merged to a single data set, they are searched in a single step that generates a single protein list.

Note This method is faster than the ProteinExtractor method (see section 7.2.2.2), but problems may occur if the combined peak list becomes too large.

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7.2.2.2 Using the ProteinExtractor to Generate a Single Protein List

►► To generate a single protein list using the ProteinExtractor

1. Start an individual ProteinExtractor search for each data set.
2. Perform a Protein List Compilation on all search results.

   This will generate a single protein list (see also sections 5.3.4.2 and 5.5.1).

The advantages of using the ProteinExtractor method for generating a single protein list are:

- Additional LC-MS/MS measurements for validating the result — for example, with Exclusion or Inclusion Scheduled Precursor Lists (SPLs) — can be easily added.
- The number of proteins identified for each individual LC-MS/MS run is provided.
- The linkage between compounds and original dataset is maintained in the result.

7.2.3 Linking Quantitation Data with Peptide Identification

Label-free quantitation data can be imported from ProfileAnalysis into the ProteinScape database.

A ProfileAnalysis project for quantitating peptides is generated for each abundance ratio between two biological states. The quantitation results are reported in a bucket table and a t-test result table.

After transfer to ProteinScape, quantitative data can be viewed by right-clicking the Project item in the Project Navigator tree and selecting **LF Peptide Quantitation > Show** from the shortcut menu.

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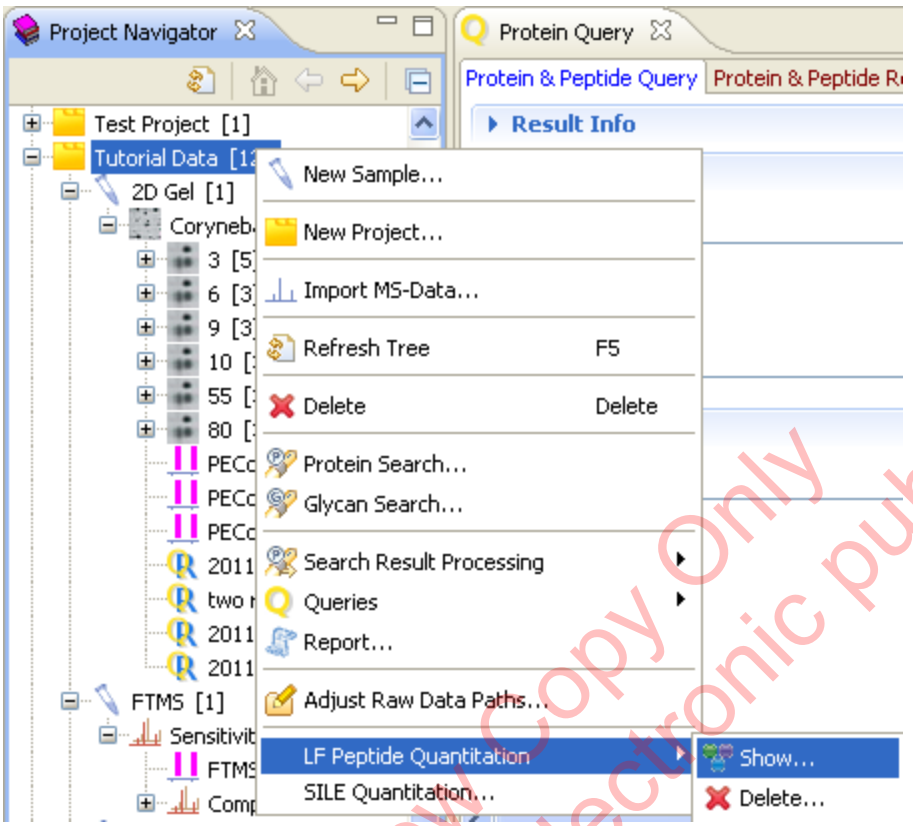

Figure 7-25 Viewing quantitative data

Select the desired ProfileAnalysis project from the **Show LF Peptide Quantitation** dialog and click **OK** to open the **LF Peptide Quantitation** table. Each row of this table contains the quantitative information for one peptide and corresponds to one bucket of the ProfileAnalysis bucket table.

Right-clicking a row in the LF Quantitation table opens a shortcut menu.

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Row	Rt [min]	MH+	Intensity	Ratio	Fold Change	p-Value	#Runs	Rank	Sequence
1	13.75	697.3972	624.67	1.07	1.07	0.6909	5		
2	13.75	1260.6408	276.42	0.91	-1.10	0.1005	6		
3	13.75	764.4504	143.46	0.97	-1.04	0.7203	4		
4	13.76	918.4744	566.45	0.99	-1.01	0.9899	6		
5			5	1.16	1.16	0.2812	8		
6			1	0.94	-1.07	0.8171	9		
7			3	1.00	1.00	0.9728	9	1	KAPAEPQR
8			1	0.99	-1.01	0.9656	7		
9			1	1.17	1.17	0.0521	7		
10			9	1.02	1.02	0.9296	9	1	SKEHTTEHLR
11			1	1.05	1.05	0.2887	7		
12			9	0.95	-1.05	0.7238	5		
13			1	0.98	-1.02	0.8025	7		
14	13.76	1186.5861	323.52	0.97	-1.03	0.6815	6		
15	13.76	712.3727	2299.33	0.52	-1.91	0.0335	6	1	SEIAHR
16	13.76	1063.5624	315.87	1.02	1.02	0.7621	7		

Figure 7-26 **LF Peptide Quantitation table with shortcut menu**

Table 7-1 **LF Peptide Quantitation table shortcut menu commands**

Command	Description
Show Cmpds. in Cmpd. table	Opens the Compound table showing all compounds contained in the corresponding bucket and the linked MS/MS compounds.
Open Bucket in PA	Opens the quantitation project in ProfileAnalysis and selects the corresponding bucket.
Excel Export	Exports the LF Peptide Quantitation table to Excel.
Copy to Clipboard	Copies the content of the LF Peptide Quantitation table to the clipboard.
Add to SPL	Adds this bucket to the Inclusion SPL (the Inclusion SPL View opens automatically).

The quantitation information reported in the **LF Peptide Quantitation** table can be linked to the peptides reported in the protein list. This process is started manually in the Project Navigator.

Right-click the Sample node containing the protein list and select **Linked LF Peptide Quantitation > Link MS/MS spectra to Buckets** to open the **Link LF Peptide Quantitation to Identification** dialog.

Select a ProfileAnalysis quantitation result (multiple selections are permitted) and select the **Use retention time correction** check box (recommended). The retention time

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correction algorithm requires that MS peak lists are exported together with MS/MS peak lists of LC-MS/MS runs (see tutorial for details). If the retention time correction is not applied, **Max. retention time deviation**, **Max. mass deviation** and **Peptide charge** values must be entered manually to perform the linkage.

Select **Use p-value for quantitation assessment** to include only those peptides whose peptide t-test value (p-value) is below the given value in the protein abundance ratio calculation. Using this option enables filtering results for significantly regulated proteins.

Click **OK** to start the linkage process between quantification and identification.

The result of the linkage between quantitation and identification can be viewed by right-clicking the Sample or data set containing the MS/MS search result and selecting **Linked LF Peptide Quantitation > Show Links**.

The **Show linked LF Peptide Quantitation** dialog opens. Select the desired LF peptide quantitation and click **OK** to open the linked results in the **Linked LF Peptide Quantitation** table.

To remove specific links, select and right-click one or more rows and select **Delete Link(s)**.

To review the details of a specific link, select and right-click a single row and select **Show Bucket in LF Peptide Quantitation table**.

►► To remove an existing link between a peptide quantitation table and LC-MS/MS runs:

1. Right-click the Sample node containing the LC-MS/MS run in the Project Navigator tree.
2. Select **Linked LF Peptide Quantitation > Delete Link**.

   The **Delete Links between LF Peptide Quantitations and Identifications** dialog will open.

3. Select the linkage(s) to be removed.
4. Click **OK** to remove the linkage(s).

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7.2.4 Quantitating Proteins

After quantitative information is linked to peptide identification information, abundance ratios of proteins can be calculated.

Right-click on the Search Result in the Project Navigator tree and select **LF Protein Quantitation > Calculate**.

The **LF Protein Quantitation** dialog opens. Select a peptide quantitation and click **OK** to calculate protein abundance ratios. The **Dynamic Range** parameter defines the maximum value for an abundance ratio detected in an experiment. This setting serves as a filter for very large abundance ratios, which may result from peak-picking artifacts.

The result is displayed in the Proteins & Peptides table of the search result. In addition to quantitative information (see section 7.1.2), the peptide part of the Proteins & Peptides table displays the number of linked buckets and the p-value of the t-test calculated in ProfileAnalysis for each peptide. The number of buckets linked to a particular peptide should not exceed one.

To remove a protein quantitation calculated for a particular abundance ratio:

1. Right-click the Search Result in the Project Navigator tree and select **LF Protein Quantitation > Remove**.
2. Select the ProfileAnalysis project containing the abundance ratio definition to be removed from the Protein & Peptide table.

7.2.5 Generating an Inclusion Scheduled Precursor List (SPL)

Note The functionality described in this section is available only if Mascot is used as search engine for peptide identifications, and only for label-free quantitation results.

Additional targeted LC-MS/MS measurements can be performed on proteins of interest contained in the protein list. These measurements can be used to improve and validate identification and quantitation results.

To find targets that can increase sequence coverage and add quantitative evidence for abundance ratios, proteins of interest are theoretically digested. The LC-MS runs from

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which a quantitative result was derived are searched for the presence of theoretical peptides that have not yet been identified.

If an *m/z* value from the LC-MS runs matches a theoretical peptide *m/z* value, this value and its retention time, are added to the inclusion scheduled precursor list (SPL).

►► To generate an inclusion SPL

1. Select and right-click the protein(s) of interest in the Protein & Peptide table.
2. Select **Generate SPL from theor. Digest** from the shortcut menu.

   The **SPL generation based on theoretical protein digest** dialog opens.

1. Select a peptide quantitation and defined values for mass and retention time tolerances.

   ProteinScape offers default tolerances. Because they are usually derived from corrected and recalibrated data, these values should be slightly increased by the user.

2. Click **OK** to close the dialog and calculate the inclusion SPL.

Commands in the Inclusion SPL Content table shortcut menu enable removal of SPL entries or export of the content to Excel.

The SPL must be saved before it can be used. Click the disk button ( ) in the Inclusion SPL Content table toolbar to open the **Save Inclusion SPL** dialog. Go to the folder where the SPL should be saved and click **Save**.

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8 Identifying Glycans and Glycopeptides

The data and parameters used for each glycan search are selected in a similar way to that used for protein searches.

Compound spectra used in glycan searches can be classified according to parameters associated with glycan search methods (see section 8.1). This classification helps users to focus on compounds of interest when searching large data sets.

8.1 Classifying Compounds

Spectra can be classified on the basis of several diagnostic features of MS/MS spectra, such as specific fragment signals, signal distances, and particular signal patterns.The parameters and protocol configurations used to perform the classification are defined in the **Spectra Classification Parameters** dialog (see Figure 8-1).

Note The parameters are divided into groups that are relevant to the type of experiment (ESI or MALDI) and class of compound being analyzed (glycopeptide or glycan).

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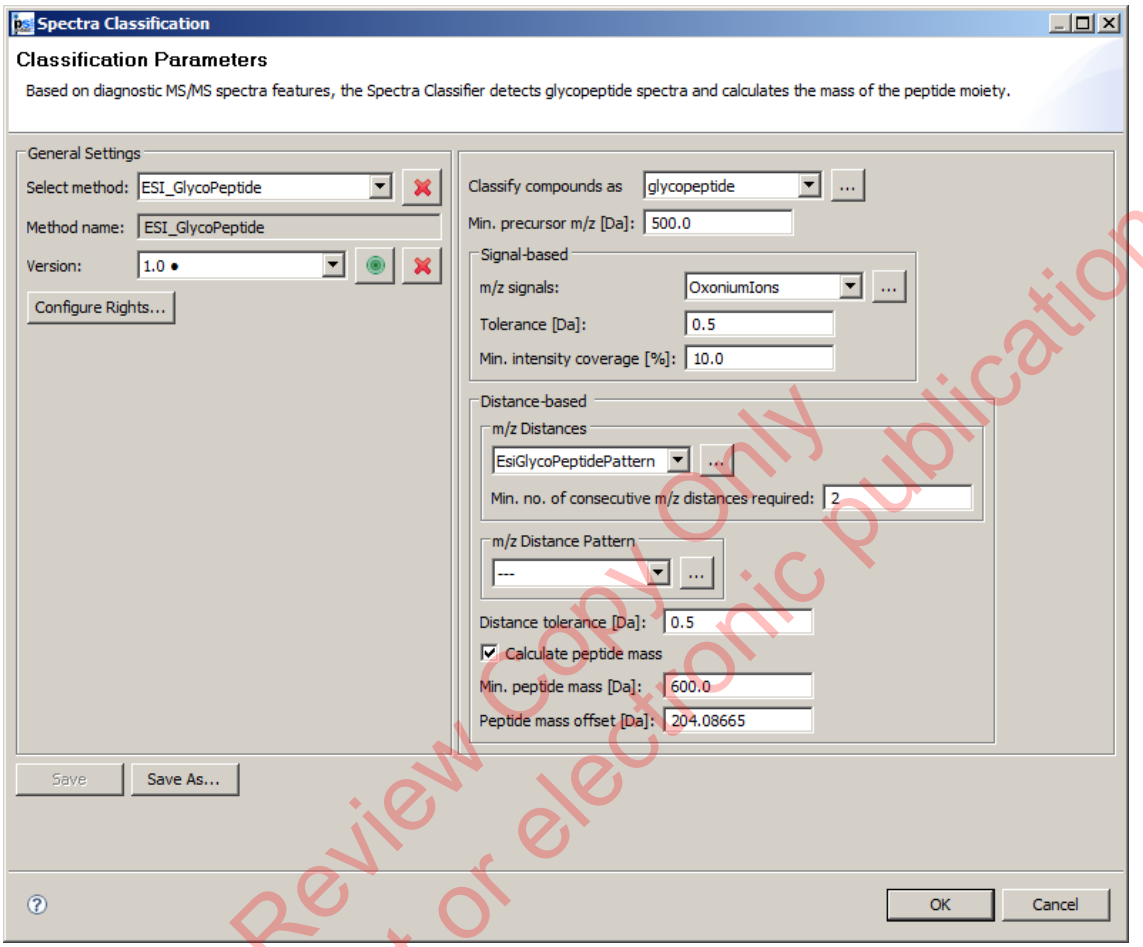

Figure 8-1 Spectra Classification dialog

General Settings

The glycan search method and version are selected in this area.

Classification & Peptide Mass Determination

- **Classify Compounds as** — The classification assigned to spectra that meet the criteria defined in the parameters above. This classification is listed in the **Class** column of the compound table.

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- **Min. precursor m/z** — Only spectra whose precursor mass exceeds this threshold are analyzed.

Signal-based classification

- **m/z signals** — Sets of MS/MS signal masses[1] characteristic for specific fragments can be defined and selected in this area. For glycans, these would be the oxonium ions observable in the low mass–range. Lists of signals for positive- and negative-mode MS/MS spectra of glycans and glycopeptides are predefined.
- **Min. intensity coverage** — This intensity coverage is calculated in the low mass–range, and determined by the highest *m/z* value in the selected list of *m/z* signals. If the intensity coverage exceeds the specified threshold the Compound is positively classified.

Distance-based classification

Fragmentation of a glycan structure leads to specific mass distances, usually corresponding to the loss of mono- or disaccharides. These distances can be usesd as a diagnostic feature for a positive classification of glycan or glycopeptide spectra.

In ESI CID spectra, the succession of mass distances can be followed down to a specific moeity. In the case of some O-linked glycopeptides, this is the peptide moiety (the aglycon). In the case of N-linked glycopeptides, this is the peptide moiety with one attached monosaccharide (HexNAc). In MALDI-TOF MS/MS, a characteristic pattern of four fragment masses can be observed for most N-linked glycopeptides.

Both strategies — the search for successive mass distances and the search for the particular MALDI TOF MS/MS pattern — can be applied during classification.

- **m/z Distances** — A series of characteristic m/z distances can be defined and selected in this area. A list of distances for ESI MS/MS spectra of N-linked glycopeptides is predefined.
- **Min. no. of consecutive m/z distances required** — Spectra containing at least this many *m/z* distances will be positively classified.

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- **m/z distance pattern** — a defined pattern of consecutive *m/z* differences[1] can be defined and selected in this area. The *m/z* patterns for MALDI-TOF MS/MS spectra of N-linked glycopeptides with and without a core fucose are predefined.
- **Distance tolerance** — *m/z* tolerance for matching the list of distances to the MS/MS spectra.
- **Calculate peptide mass** — Select this option to calculate the mass of the peptide moiety in glycopeptides. Clear this option when analyzing glycans.
- The corresponding **peptide mass offset** can also be defined. For instance, an offset of 204.08665 reflects the mass difference of a glycopeptide carrying a HexNAc glycan. Note that this offset is expected to produce the neutral mass of the peptide moiety of a glycopeptide. For MALDI data of N-glycans and for ESI data of O-glycans the offset is 1.00728.

►► To classify compounds

1. Right-click a Combined Data node ( ) in the Project Navigator tree and select **Classification**.

   The **Specta Classification Parameters** dialog opens.

2. Either:

   a. Select an existing glycan search method and classification type and click **Start** to start classification.

OR

   b. Define new classification parameters and save the settings to a new glycan search method (click **Save As**) or a new version of an existing method (click **Save**). Click **Start** to start classification.

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[1] *Configurations for this parameter are created and edited in the same way as Protocol Navigator node attributes (see section 3.7.3).*

►► To edit a classification type

1. Select **Edit > Methods > Classification** to open the **Spectra Classification** dialog.

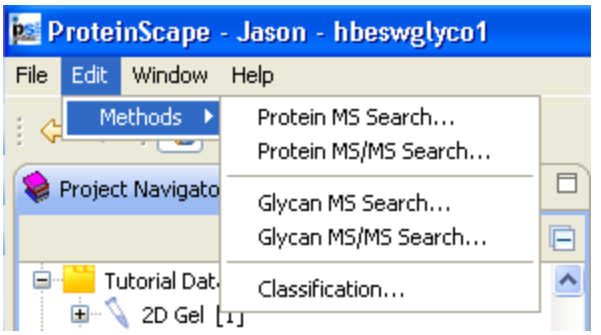

Figure 8-2 Edit > Methods menu

3. Define new classification parameters and save the settings to a new glycan search method (click **Save As**) or a new version of an existing method (click **Save**).
4. Click **OK** to close the dialog.

   The most recently created version of a glycan search method is automatically set as the standard (default) version in the **Version** drop-down list (indicated by a closed circle next to the version name).

►► To create a classification type

1. Select **Edit > Methods > Classification** to open the **Spectra Classification** dialog.

5. Click the Browse button to the right of the **Classify compounds as** drop-down list.
6. Click **Save As** and type a name into the **New method name** field.
7. Define new classification parameters and save the settings to a new glycan search method (click **Save As**) or a new version of an existing method (click **Save**).
8. Click **OK** to close the dialog.

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8.2 Defining Data and Parameters used for Glycan Searches

►► To define data and parameters used for glycan searches

1. Right-click a data or non-data (Project, Sample, LC, Gel, or Digest) node containing the desired spectra and select **Glycan Search** from the shortcut menu.

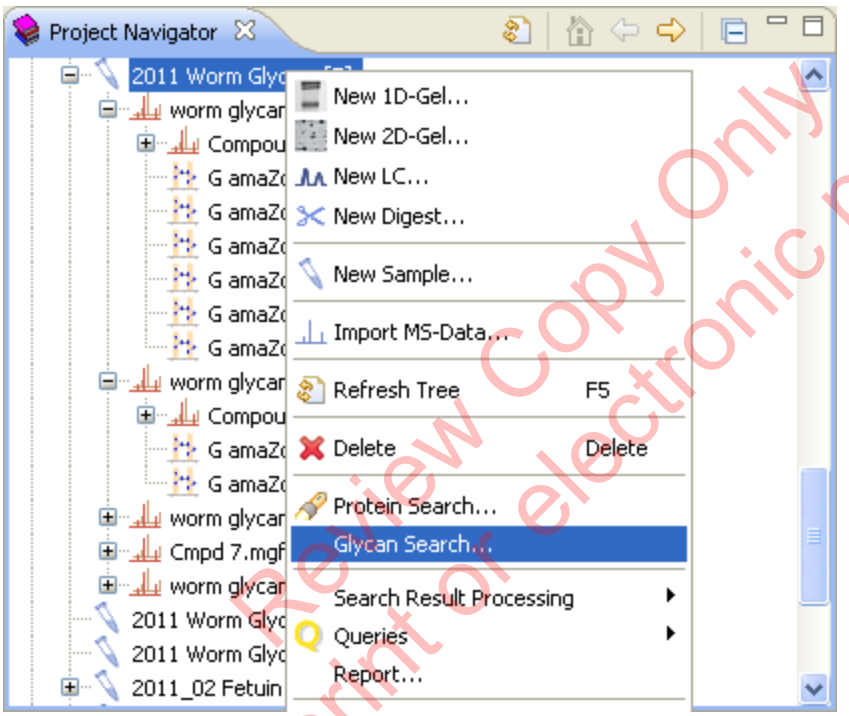

Figure 8-3 Starting a Glycan Search using the Project Navigator shortcut menu

The **Glycan Searches > Spectra Selection** dialog box opens.

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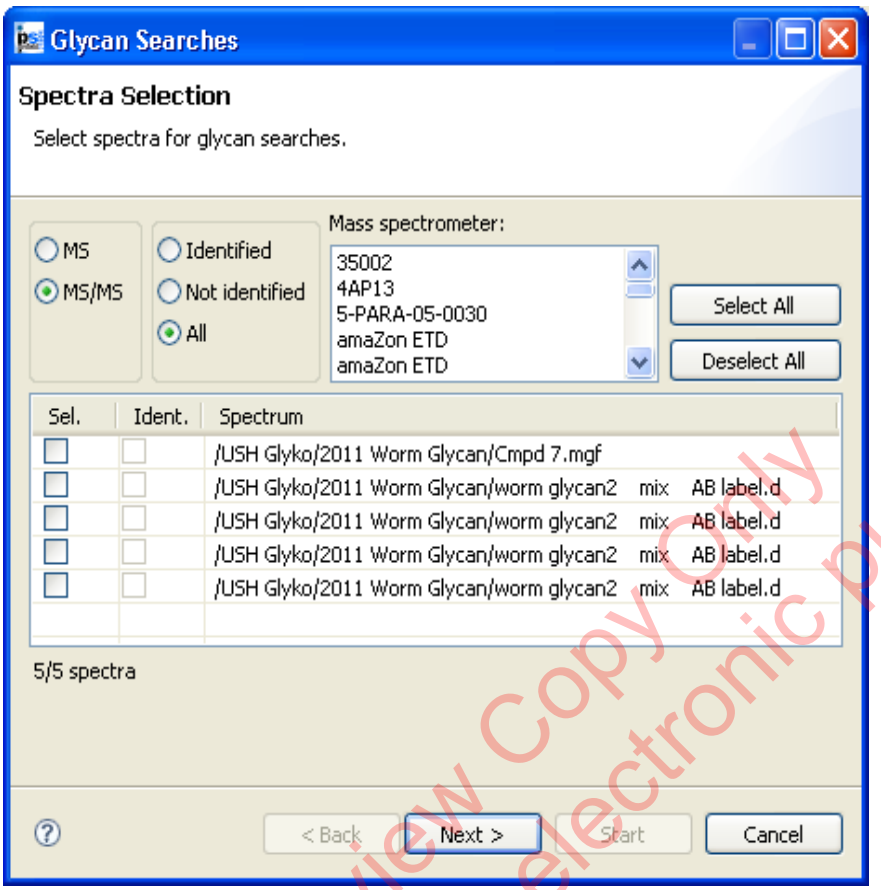

Figure 8-4 Glycan Searches > Spectra Selection dialog

1. Select the relevant data type (**MS (PMF)** or **MS/MS (PFF)**) and identification status (**Identified**, **Not identified** or **All**).

   The list of data sets can be filtered by selecting one or more mass spectrometers from the **Filter by mass spectrometer** list.

3. In the **Sel.** column, select the box next to the data that will be used for the glycan search. Use **Select All** or **Deselect All** to include or remove all data sets from the selection.
4. Click **Next**.

   The **Glycan Searches > Search Parameters** dialog opens.

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Use the **< Back** and **Next >** buttons in the lower-right corner of the dialog box to navigate between the dialog box pages. Click **Start** to begin the database search and **Cancel** to exit the dialog box.

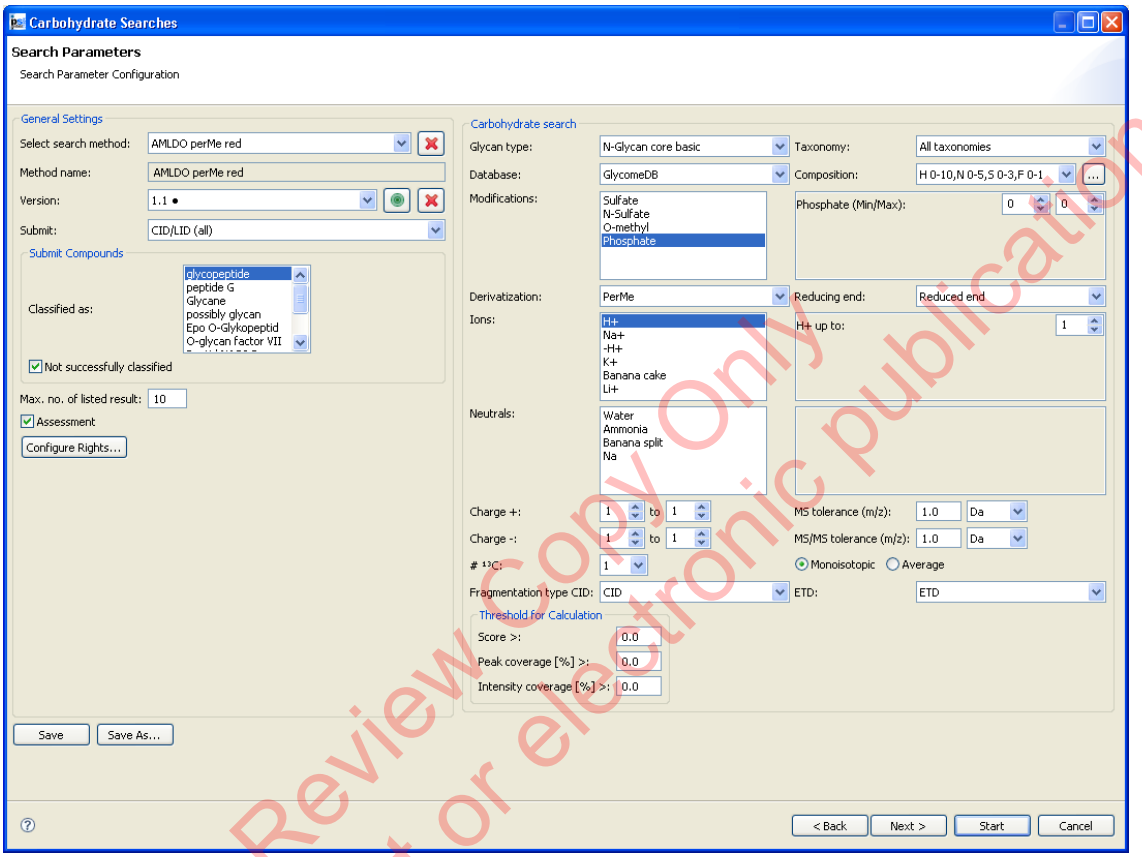

Figure 8-5 Glycan Searches > Search Parameters dialog

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8.3 Glycan Search Methods

A glycan search method defines the parameters used to identify glycans or glycopeptides in ProteinScape.

The glycan search method defines the glycan database used, modifications that should by taken into account, the charge on fragments, mass tolerances, and enables filtering of results according to glycan score.

8.3.1 Using Existing Glycan Search Methods

►► To use an existing glycan search method for a glycan search

1. Select an existing search method from the **Select search method** drop-down list.
2. Select the desired method version from the **Version** drop-down list.
3. Click **Start** to start the glycan search.

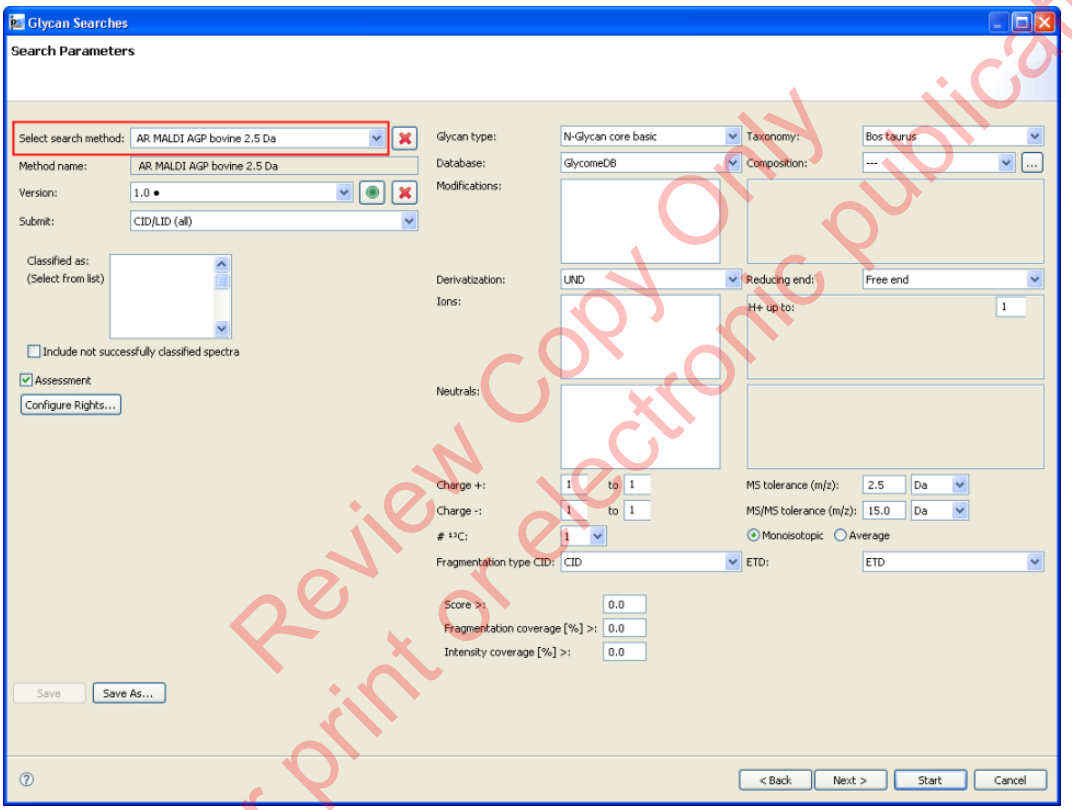

Figure 8-6 Glycan Searches > Search Parameters dialog

8.3.2 Editing and Creating Glycan Search Methods

Glycan search methods cannot be changed after they are created and saved to the ProteinScape database. Any changes to an existing method can be saved as an updated version of the existing method or as a new method.

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►► To create a new version of an existing glycan search method

1. Select **Edit > Methods > Glycan MS(MS/MS) Search** to open the **Glycan Searches** dialog.

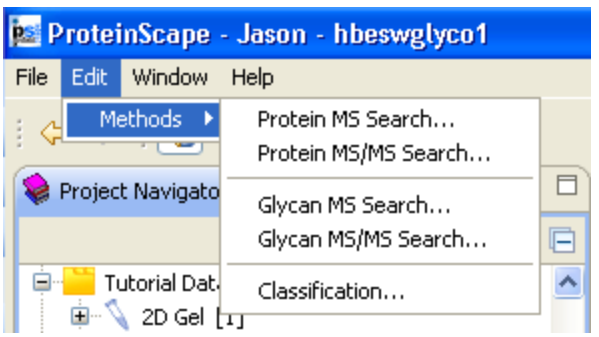

Figure 8-7 Edit > Methods menu

2. Select an existing search method from the **Select search method** drop-down list.
3. Make the desired changes to the search parameters and assessment configuration in the **Glycan Searches** dialog pages.
4. Click **Save** and type a new version name in the **Save Method** dialog.
5. Click **OK** to close the dialog.

   The most recently created version of a glycan search method is automatically set as the standard (default) version in the **Version** drop-down list (indicated by a closed circle next to the version name).

►► To create a new glycan search method

1. Select **Edit > Methods > Glycan MS(MS/MS) Search** to open the **Glycan Searches** dialog.
2. Make the desired changes to the search parameters and assessment configuration in the **Glycan Searches** dialog pages.
3. Click **Save As** and type a method name and first version name in the **Save As New Method** dialog.
4. Click **OK** to close the dialog.

   The new glycan search method is added to the **Select search method** drop-down list.

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8.4 Glycan Searches Dialog

8.4.1 General Settings

The following parameters are set in the **General Settings** area of the Glycan Searches Parameter Configuration window.

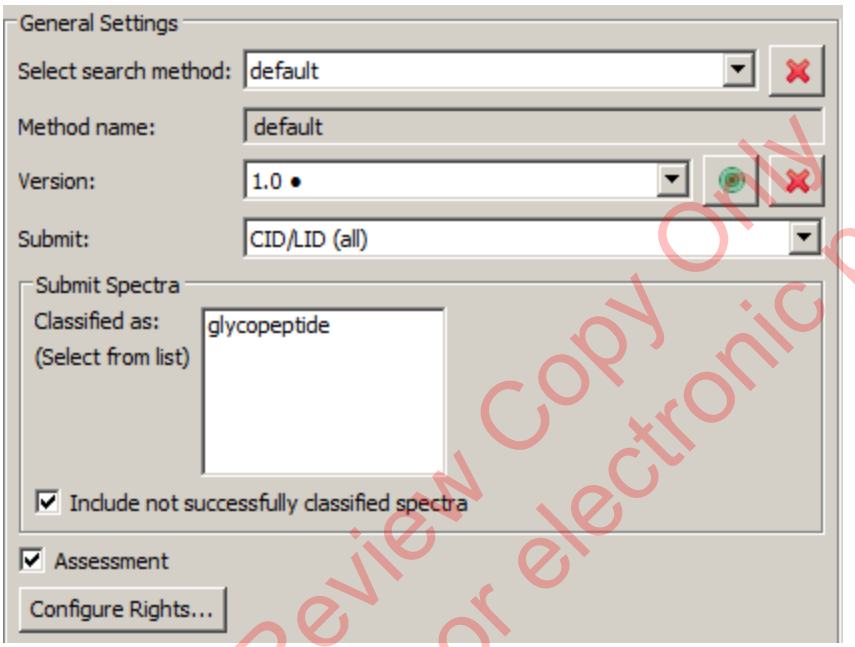

Figure 8-8 General Settings area of the Glycan Searches Parameter Configuration window

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Table 8-1 General Settings parameters

Parameter	Comments
Select search method	Select an existing search method from the drop-down list. To delete a method, select it in the list and click .
Method name	Displays the selected search method.
Version	Select a version using the drop-down list. Click to define the selected method version as the default version. To delete a version, select it in the list and click .
Submit	Select to submit **All**, **CID/LID**(all or positive or negative), or **ETD/ECD** spectra.
Submit spectra	
Classified as	Compounds with the selected classification(s) will be submitted for analysis (see section 8.1). Select multiple entries by pressing the **CTRL** key during selection.
Include not successfully classified spectra	Select to include unclassified spectra for analysis. Clear to submit only classified spectra for analysis.
Assessment	Perform automatic glycan assessment (see section 8.4.3).
Configure Rights	Launch the Configure Method Access window.

8.4.2 Glycan Search Parameters

The area to the right of the **General Settings** displays the **Glycan search** parameters. Configurations for the parameters **Taxonomy**, **Composition** and **Fragmentation type** can be created (see section 3.7.3) and edited (see section 3.7.2) through the Protocol Navigator. See the *ProteinScape Administrator's Manual* for information on how to introduce new parameters.

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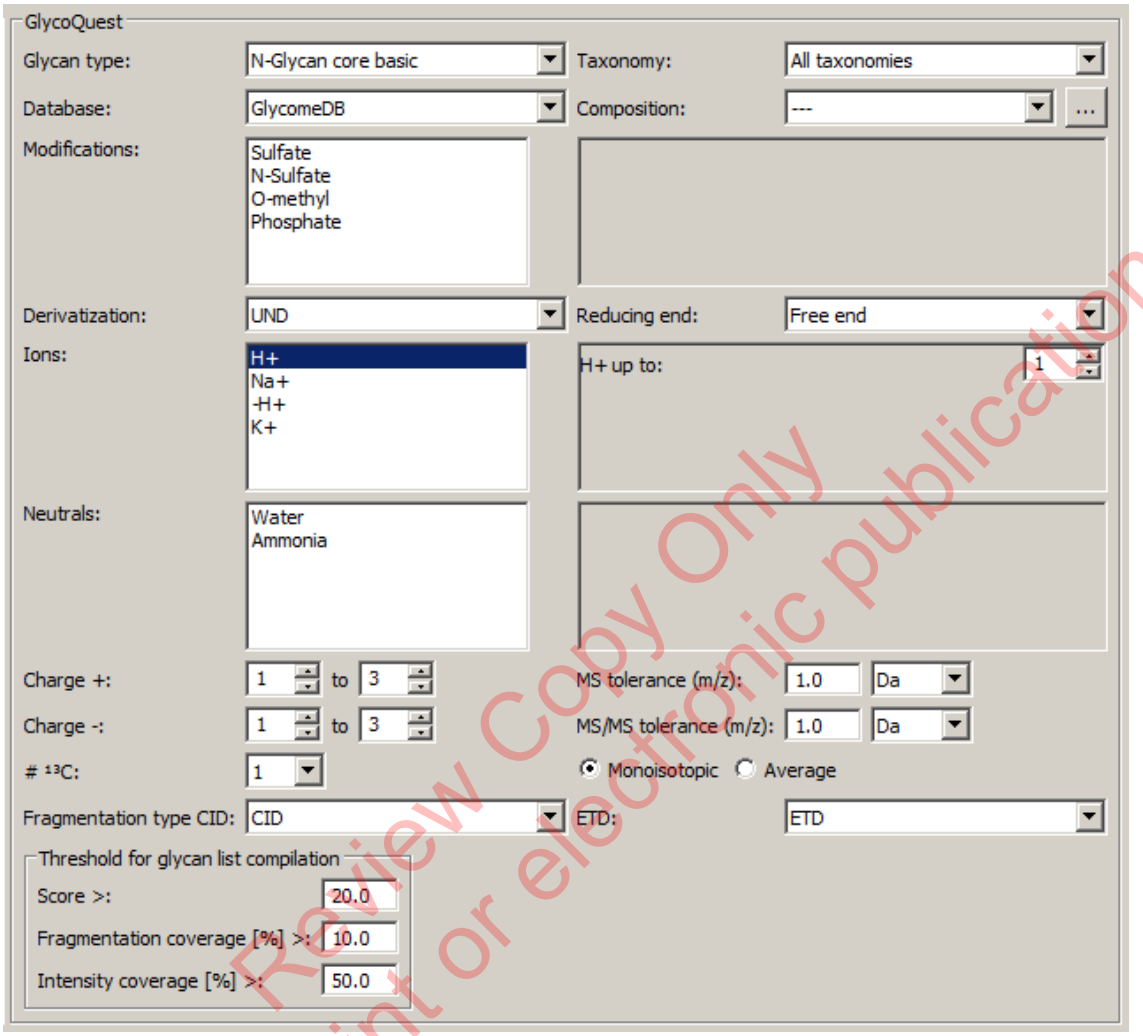

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Figure 8-9 Glycan search parameters

Parameter	Description
Glycan type	Select a glycan type from the drop-down list. If the glycan type is unknown select the option **---** at the top of the list.
Taxonomy	Search the selected taxonomy.
Database	Select a glycan database to be used for the search (see section 8.4.2.1).

Parameter	Description
Composition	Select a defined composition from the drop-down list or click the **Browse** button to edit or create a composition.
Modifications (Min/Max)	Select desired modification(s) from the drop-down list. Select multiple modifications by holding **SHIFT** (consecutive entries) or **CTRL** (individual entries) when making selections. Enter the desired minimum and maximum number of modifications in the relevant boxes.
Derivatization	Select a glycan derivatization reagent from the drop-down list.
Reducing end	Select a reducing end chemical group from the drop-down list.
Ions (up to)	Select an ionic species from the drop-down list and the number of ions present.
Neutrals (Loss/Gain)	Select a neutral species from the drop-down list and the respective number of atoms/molecules lost or gained.
Charge +	Define the charge range of positively charged species included in the search.
Charge –	Define the charge range of negatively charged species included in the search.
MS tolerance (*m/z*)	Define the *m/z* tolerance for MS searches (%, Da, mmu or ppm).
MS/MS tolerance (*m/z*)	Define the *m/z* tolerance for MS/MS searches (%, Da, or mmu).
# ^{13}C	Define the number of carbon-13 atoms.
Monoisotopic/Average	Use monoisotopic or average mass.
Fragmentation type	Define fragmentation type used in the experiment.
Threshold for calculation	
Score >	Define minimum score for inclusion in glycan list.
Fragmentation Coverage [%] >	Define minimum fragmentation coverage for inclusion in glycan list.

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Parameter	Description
Intensity Coverage [%] >	Define minimum intensity coverage for inclusion in glycan list.

8.4.2.1 Glycan Search Databases

ProteinScape performs glycan searches on a local copy of the GlycomeDB (*www.glycome-db.org*), a meta-database that is frequently updated with structures from a number of individual glycan databases[1].

Alternatively, a user-defined database containing a selected subset of structures or novel structures (see section 8.6.4.5) can be created.

Glycan search database synchronization and creation of user-defined databases requires Administrator rights. See the *ProteinScape Administration Manual* for further details.

8.4.2.2 Creating and Editing Custom Glycan Databases

Note Administrator rights are required to create, edit and delete user-defined databases.

►► To create a custom database

1. Select **Window > Admin Preferences > Processing Kernel > Glycan Searches > Glycan Search Databases** to open the **Glycan Search Databases** dialog.
2. Click **New DB** and type a **Name** and **Abbreviation** into the respective fields.
3. Click **OK** to create the new database.

►► To edit the name and abbreviation of an existing custom database

1. Select **Window > Admin Preferences > Processing Kernel > Glycan Searches > Glycan Search Databases** to open the **Glycan Search Databases** dialog.

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[1] *The Kyoto Encyclopedia of Genes and Genomes (KEGG) may be used for academic purposes only. A license must be obtained for commercial use. The initial ProteinScape installation does not contain the glycan structures from the KEGG database. Users must read and accept the terms of use (www.genome.jp/kegg/legal.html) before synchronizing the ProteinScape database to include structures in the KEGG database.*

2. Select an existing database and click **Edit DB**.
3. Make the desired changes to the **Name** and **Abbreviation** fields.
4. Click **OK** to save the changes.

►► To delete a custom database

1. Select **Window > Admin Preferences > Processing Kernel > Glycan Searches > Glycan Search Databases** to open the **Glycan Search Databases** dialog.
2. Select an existing database and click **Delete DBs**.
3. Click **OK** to delete the database.

8.4.3 Glycan List Assessment

The user can decide which of the glycans found in the search should be accepted.

This can be done either manually or by selecting **Assessment** in the **General Settings** and defining a minimum score threshold on the **Assessment** page of the **Glycan Searches** dialog. This threshold will be automatically applied to the glycan list.

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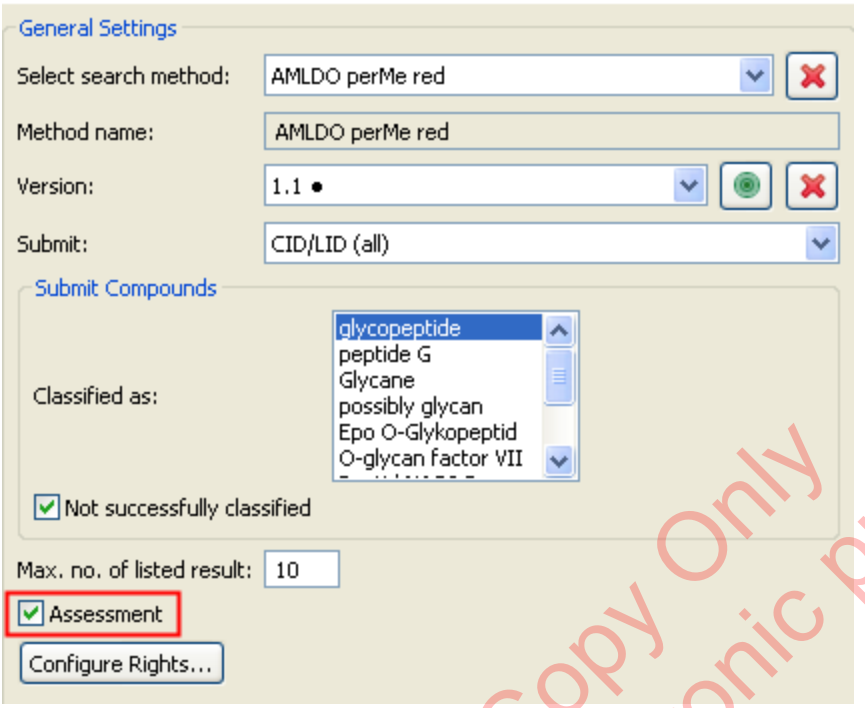

Figure 8-10 **Selecting the Assessment feature in the General Settings area of the Glycan Searches dialog**

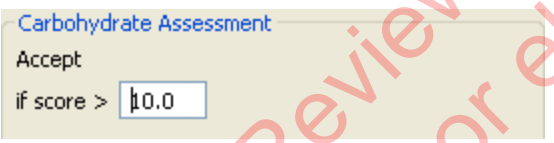

Figure 8-11 **Glycan and Peptide Assessment areas of the Glycan Searches Assessment Configuration dialog**

8.5 Starting and Repeating Glycan Searches

A glycan search is started by clicking **Start** on either of the **Glycan Searches** dialog pages.

To repeat a glycan search, select **Repeat Glycan Search** in the shortcut menu of an existing **Search Result** node, or **Repeat Search** in the **Info > Search Result Info** area of its Main View.

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Selecting either of the above options for repeating a search opens the **Glycan Searches Parameter Configuration** dialog box where any search parameter can be changed. Click **Start** to begin the new search.

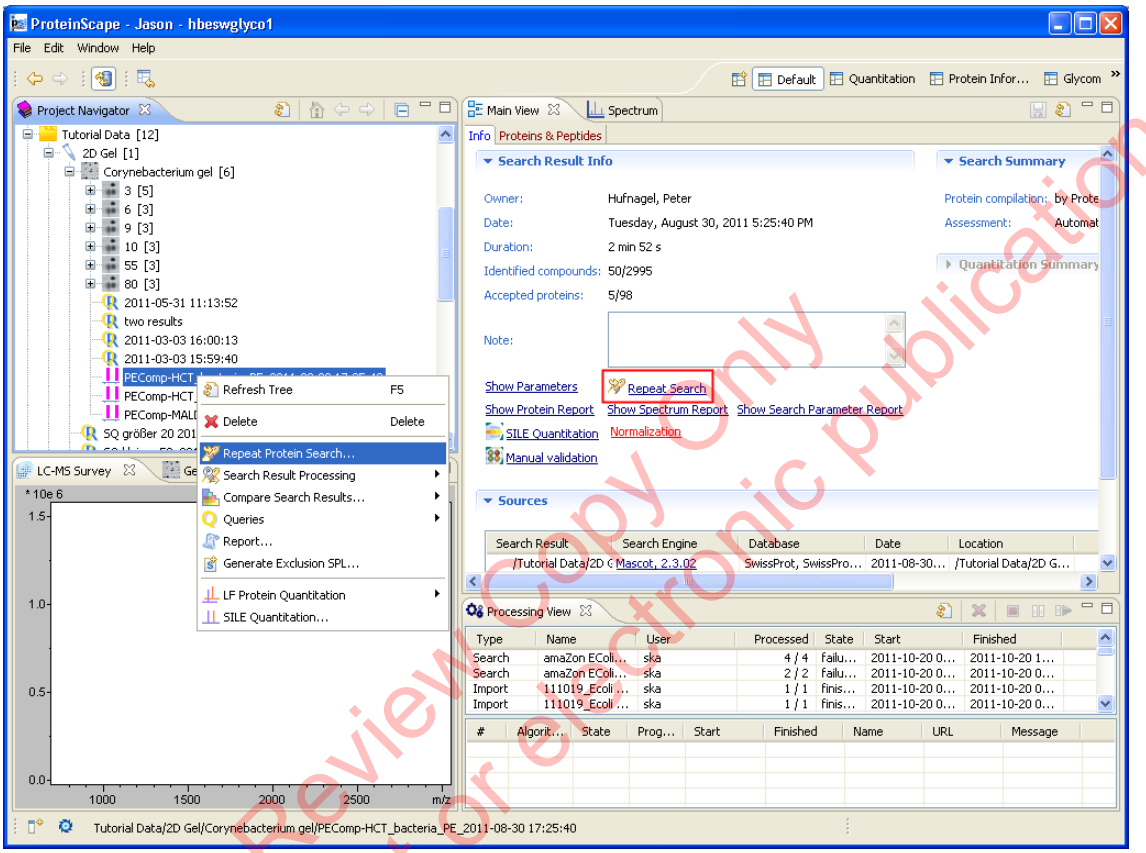

Figure 8-12 **Repeating a glycan search using (left) the shortcut menu or (right) Repeat Search in the Main View**

8.6 Evaluating and Displaying Glycan Search Results

The Main View of a glycan **Search Result** node consists of two pages:

- Info page
- Glycans & Fragments page

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Clicking a tab in the upper-left corner of the Main View moves the respective page into the foreground (see Figure 8-13).

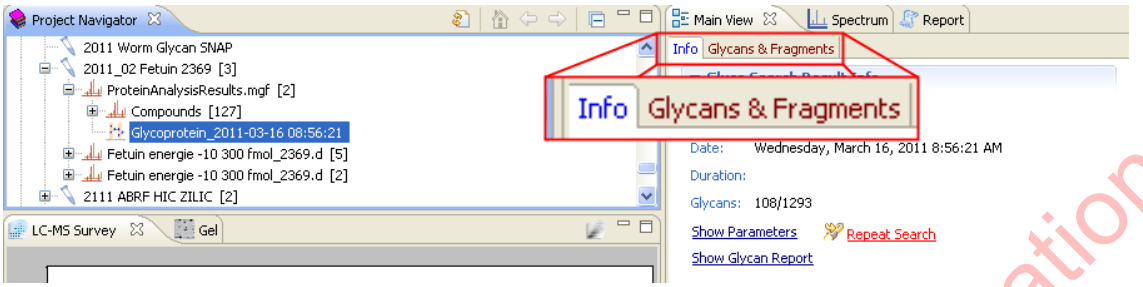

Figure 8-13 Info and Glycans & Fragments page tabs in a Glycan Search node Main View

8.6.1 Glycan Search Info Page

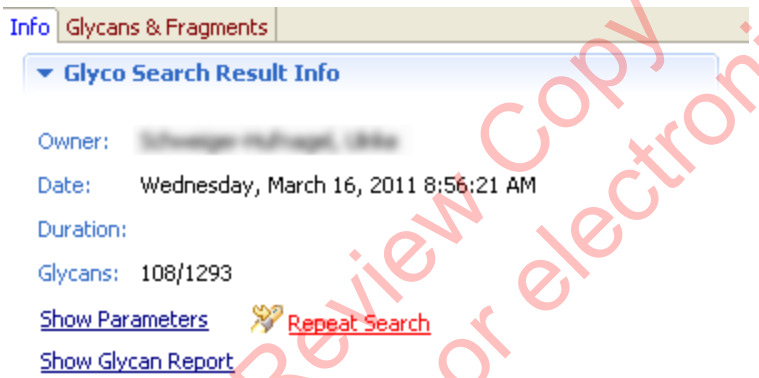

Figure 8-14 Info page of the glycan Search Result Main View

The **Glyco Search Result Info** area of the glycan Search Result Main View **Info** page displays:

- **Owner**: — the project owner
- **Date**: — date and time that the search was started
- **Duration**: — time required for the search
- **Glycans**: — the number of identified compounds

It also contains buttons for additional actions.

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Table 8-2 Additional action buttons on the Glycan Search Info page

Action	Description
Show Parameters	Displays the **Search Parameter** dialog box pages with the parameters used for the search. Parameters cannot be altered.
Repeat Search	This button is active if the **Search Method** applied has been saved. Clicking this button opens the **Search Parameter** dialog box, where parameters can be changed and a new search performed.
Show Glycan Report	Displays a detailed report of all accepted glycans (see section 10.2).

8.6.2 Glycans Search Glycans and Fragments Page

The **Glycans & Fragments** page of the glycan **Search Result** Main View displays information on the identified glycans and fragments in tabular form.

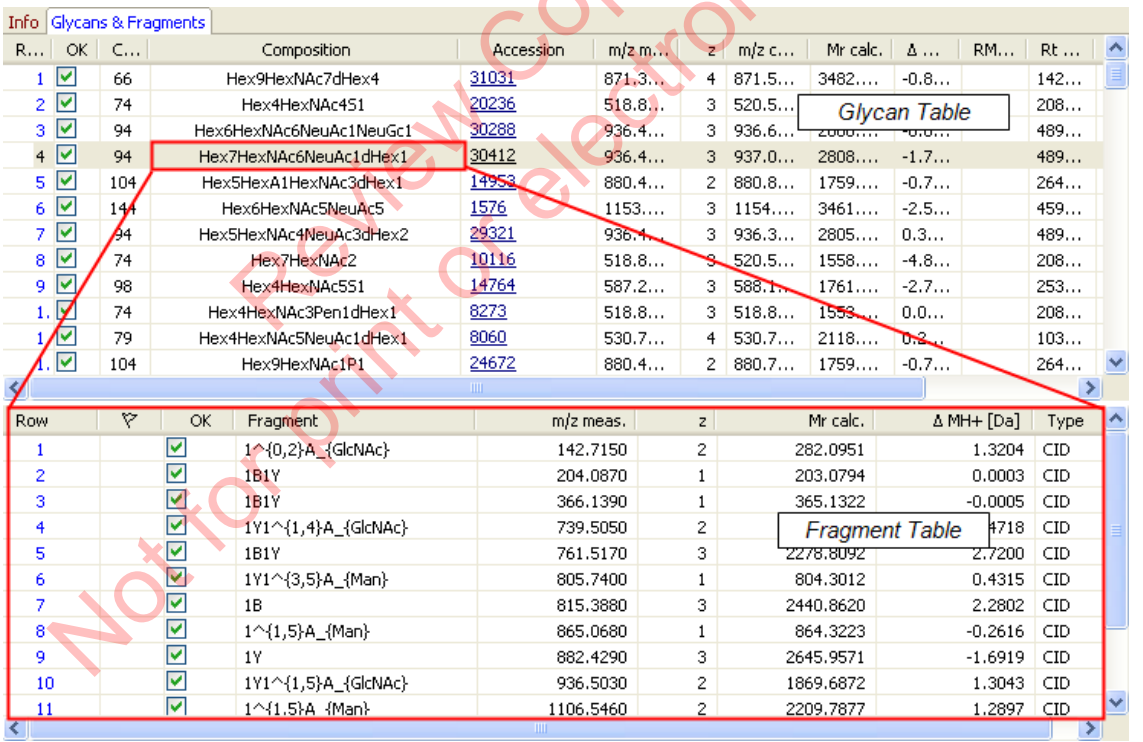

Figure 8-15 Glycans & Fragments page of the glycan Search Result Main View

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The upper part of the page displays a list of accepted glycans; the lower part displays the list of fragments identified in the selected glycan.

8.6.2.1 Glycan Table

The glycan table displays a wide range of information about the glycans accepted as having been identified in the search.

Column	Description
Rank	Ranking based on number of identified peptides.
Row	Row index
OK	Selected box indicates glycan is accepted as identified. The accepted status is automatically assigned during the search. Status can be changed manually by clearing box or using the shortcut menu.
Cmpd.	Compound number.
Composition	Glycan composition.
Accession	Glycome DB database accession number.
***m/z* meas. / calc.**	Measured /calculated mass-to-charge ratio.
z	Charge.
Mr meas. / calc.	Measured /calculated Mr value.
Δ MH+ [Da] / [ppm]	Monoisotopic mass difference.
RMS / RMS90 [Da] / [ppm]	Deviation from predicted mass (root mean square value / root mean square 90% confidence value).
Rt (min)	Retention time in minutes.
Score	Glycan score.
#Cmpds.	Number of compounds matching the same glycan.
Type	Fragmentation type.
Flag	Assign a colored flag by right-clicking a glycan entry and selecting **Flags** from the menu.
Visible	Show/Hide entry in Glycans & Fragments table. Entries hidden here can be found in the Alternative Glycans table.

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Column	Description
MH+ meas. / calc.	Measured /calculated MH+ value.
Δ *m/z* [Da] / [ppm]	Difference in mass-to-charge ratio.
Int.	Precursor ion intensity.
IntCov. [%]	Intensity coverage (%).
#AltG	Number of alternative glycans.
Location	Location of source file.

8.6.2.2 Fragment Table

The fragment table displays information about the accepted fragments identified in the glycan selected in the glycan table.

Column	Description
Row	Row index
Flag	Assign a colored flag by right-clicking a fragment entry and selecting **Flags** from the menu.
OK	Selected box indicates fragment is accepted as identified. The accepted status is automatically assigned during the search. Status can be changed manually by clearing box or using the shortcut menu.
Fragment	Fragment structure notation.
***m/z* meas. / calc.**	Measured /calculated mass-to-charge ratio.
z	Charge.
Mr meas. / calc.	Measured /calculated Mr value.
Δ MH+ [Da] / [ppm]	Monoisotopic mass difference.
Type	Fragmentation type.

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Column	Description
Visible	Show/Hide in Glycans & Fragments table. Entries hidden here can always been found in the Alternative Glycans table.
MH+ meas. / calc.	Measured /calculated MH+ value.
Δ *m/z* [Da] / [ppm]	Difference in mass-to-charge ratio.
Int.	Fragment intensity.
Rel. Int. [%]	Relative fragment intensity (%).
#AltF	Number of alternative fragments (matches).

8.6.3 Glycans & Fragments Table Shortcut Menu

The information displayed in the glycan or fragment table can be configured using the respective shortcut menu, which is launched by right-clicking anywhere in the table. The shortcut menu contains the common table view commands (see section 2.6). and the following additional commands.

Show

Glycan- or fragment-specific Views (see section 8.6.4) can be launched using the **Show** option in the respective menu.

Note The checkmarks in the **OK** column and flags in the glycan and fragment parts of a table are completely independent of each other. This means that a fragment can be accepted (**OK** selected) even if the respective glycan has not been accepted. Equally, an accepted glycan can contain fragments that did not meet the acceptance criteria (**OK** not selected).

8.6.4 Glycan- and Fragment-Specific Views

Three Views can be launched from the Glycan Table menu (see Figure 8-16):

- Glycan Structure Editor View (see section 8.6.4.5)
- Glycan Fragment Summary View (see section 8.6.4.6)
- Alternative Glycans View (see section 8.6.4.1)

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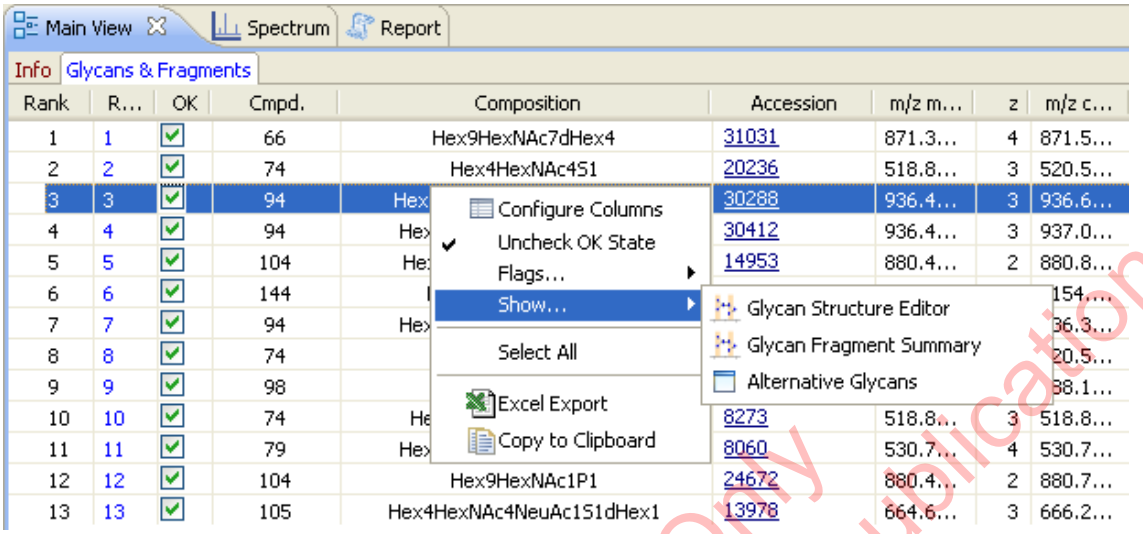
Main View
Spectrum
Report
Info
Glycans & Fragments
Rank R... OK Cmpd. Composition Accession m/z m... z m/z c...
1 1 66 Hex9HexNAc7dHex4 31031 871.3... 4 871.5...
2 2 74 Hex4HexNAc4S1 20236 518.8... 3 520.5...
3 3 94 Hex 30288 936.4... 3 936.6...
4 4 94 Hex 30412 936.4... 3 937.0...
5 5 104 He 14953 880.4... 2 880.8...
6 6 144 154...
7 7 94 Hex 36.3...
8 8 74 20.5...
9 9 98 38.1...
10 10 74 He 8273 518.8... 3 518.8...
11 11 79 Hex 8060 530.7... 4 530.7...
12 12 104 Hex9HexNAc1P1 24672 880.4... 2 880.7...
13 13 105 Hex4HexNAc4NeuAc1S1dHex1 13978 664.6... 3 666.2...
Configure Columns
Uncheck OK State
Flags...
Show...
Select All
Excel Export
Copy to Clipboard
Glycan Structure Editor
Glycan Fragment Summary
Alternative Glycans

Figure 8-16 Views available through the Glycan Table shortcut menu

One View can be launched from the Fragment Table menu (see Figure 8-17):

- Alternative Fragments View (see section 8.6.4.2)

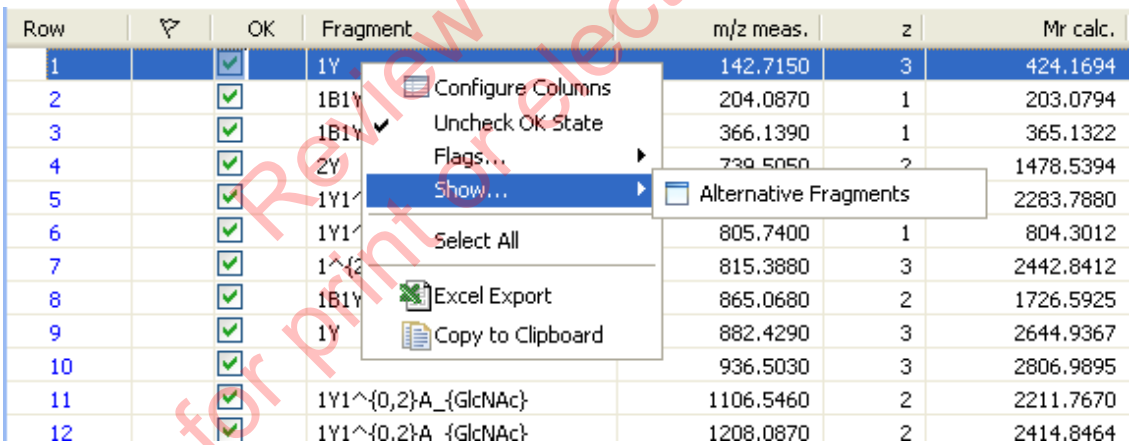

Figure 8-17 Views available through the Fragment Table shortcut menu

8.6.4.1 Alternative Glycans View

Glycan databases frequently contain a number of entries for a given mass.

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This means that a whole group of glycans may be identified for a given data sets. In such cases, the Glycan Table displays the best hit, that is, the hit with the highest score. The other, similar glycans can be accessed in the **Alternative Glycans** View.

The Alternative Glycans View is opened through the Glycan Table menu (**Show > Alternative Glycans**).

For a given glycan, the **Alternative Glycans** View shows all equivalent matches that cannot be discriminated on the basis of the underlying MS data.

In essence, the **Alternative Glycans** View is a second Glycan & Fragments table (see section 8.6.2) with the same parameters and functionality. To display the spectrum, structure or fragment summary for a glycan in the **Alternative Glycans** View, launch the shortcut menu by right-clicking a glycan and select **Show** and the respective option .

Selecting the **Visible** check box for an entry in the Alternative Glycans table makes the entry visible in the Glycans table. Clearing the **Visible** check box removes the entry from the Glycan table.

An analogous feature (Alternative Fragments View) is available for the Fragment Table (see section 8.6.4.2).

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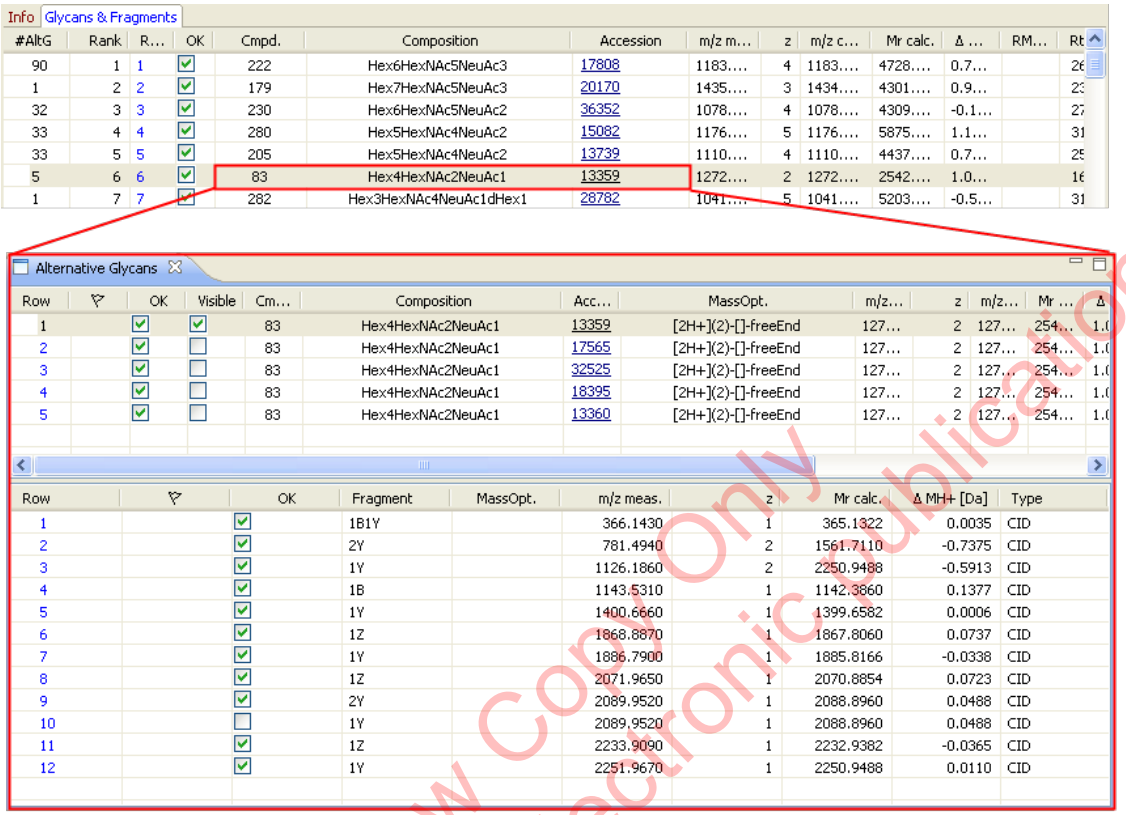

Figure 8-18 Alternative Glycans View

8.6.4.2 Alternative Fragments View

The **Alternative Fragments** View is opened through the Fragment Table menu (right-click a fragment and select **Show > Alternative Fragments**).

The Fragment table usually displays only one of all possible matches. However, there are sometimes several possible matching fragments for a given compound (indicated in the **#AltF** = number of alternative fragments column of the Fragment table).

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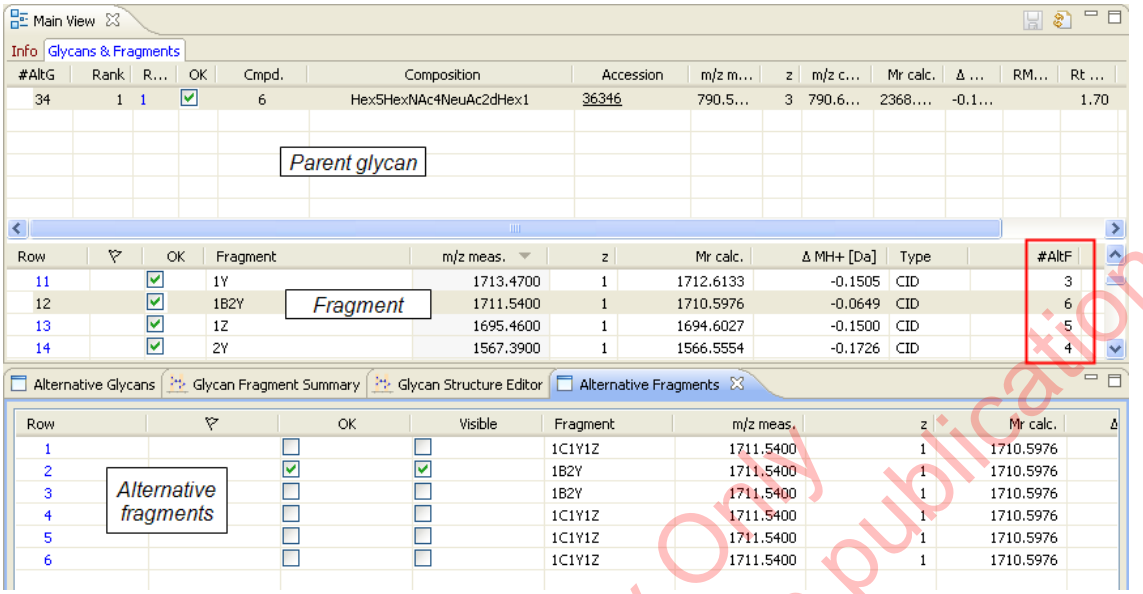

Figure 8-19 Alternative Fragments View

8.6.4.3 Glycan Structure View

The **Glycan Structure View** displays the structure of the glycan and fragment currently selected in the Glycan and Fragment table in Consortium for Functional Glycomics (CFG) format.

Click the β4 button in the view toolbar to show/hide linkage information (if data support this function).

Select **Window > Show View > Other > Glycan Structure View** to display the Glycan Structure View.

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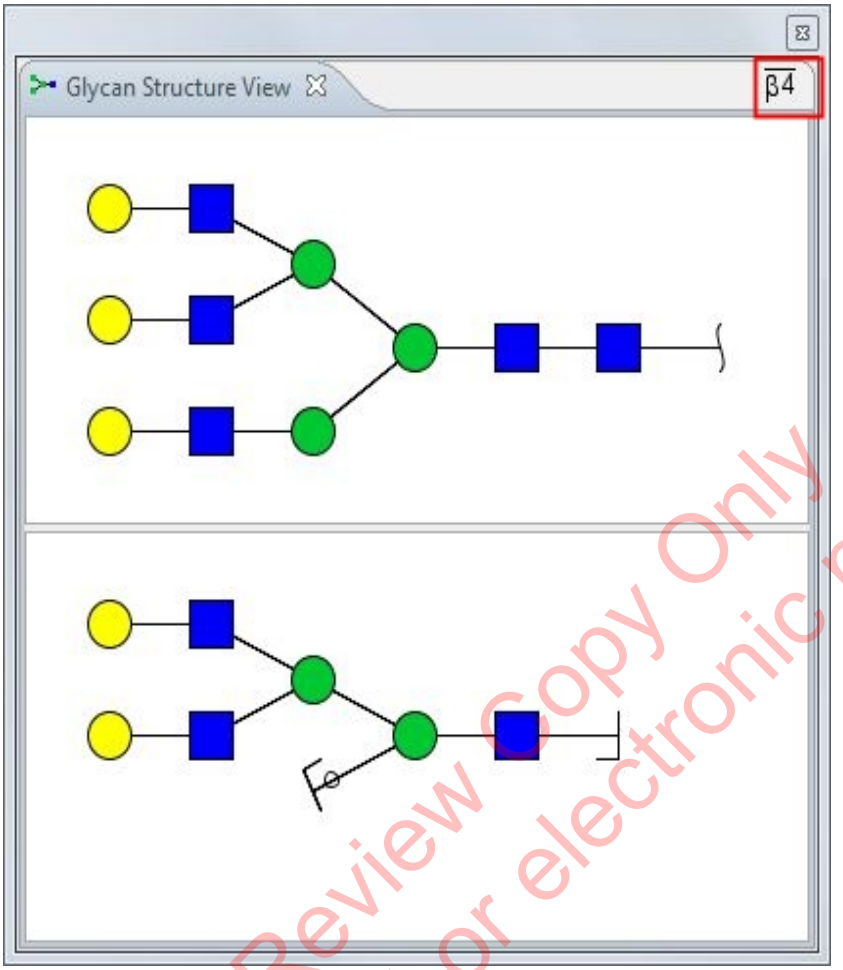

Figure 8-20 Glycan Structure View

8.6.4.4 Glycan Spectrum View

An annotated spectrum of the glycan is displayed by selecting a glycan and selecting **Window > Show View > Spectrum**.

Glycan peaks are colored green and labeled with CFG format representations of the fragment structure. Moving the mouse pointer over a CFG representation displays an annotated, magnified image in the upper left corner of the View.

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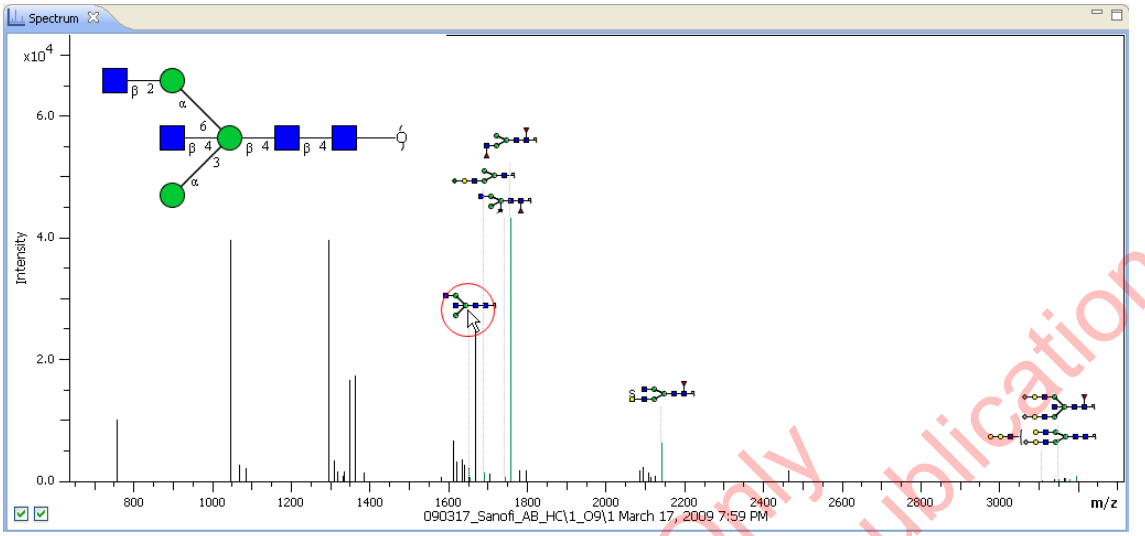

Figure 8-21 Glycan Spectrum View showing magnified fragment image in the upper-left corner

The shortcut menu offers the same options as for peptide spectra (see section 6.4.2.1). The **Preferences** option offers three additional options:

- **Image Size Percentage** — Define the peak annotation image size as a percentage of the full size
- **Magnified Image Size Percentage** — Define the size of the magnified image that appears in the upper-left corner when the mouse pointer hovers over an annotation.
- **Image Layout** — Select horizontal or vertical orientation

8.6.4.5 Glycan Structure Editor

The Glycan Structure Editor can be used to create custom glycan database entries that can be used in glycan searches. Glycan structures can be created from scratch using the Glycan Structure Editor toolbar or imported in GlycoCT or Glyde II XML format.

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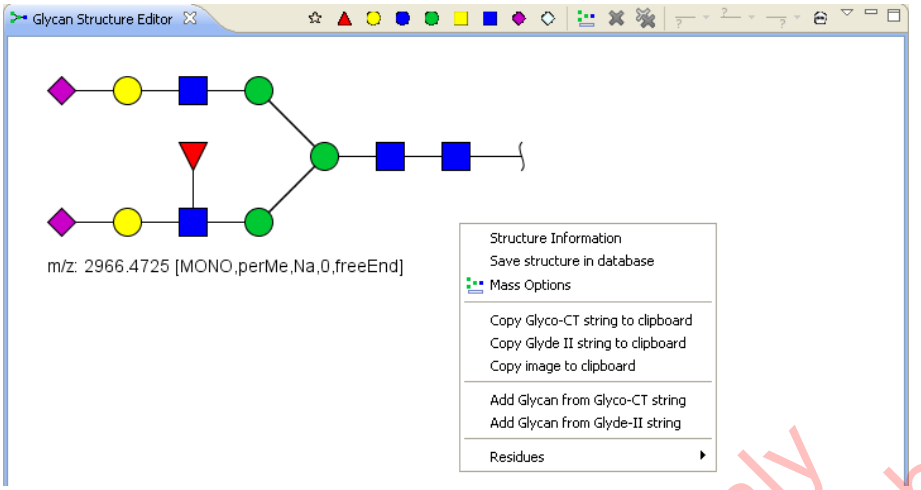

Figure 8-22 Glycan Structure Editor View with shorcut menu

►► To create entries for a custom glycan database

1. Create a glycan structure by either:

   a. Adding components using the Glycan Structure Editor toolbar (see Table 8-3).

   OR

   b. Right-clicking the View, selecting **Add Glycan from Glyco-CT/Glyde II string**, and pasting an XML description of the structure into the **Import Glyco-CT/Glyde II String** dialog. Click **OK** to generate the structure from the XML description.

►► To add entries to a custom glycan database

1. Select and right-click the glycan structure and select **Save structure in database**.
2. Select a database and type a name in the **Add Glycan to Database** dialog and click **OK**.
3. A confirmation message appears.

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Table 8-3 Glycan structure editor toolbar buttons

Button	Name	Action
	Xyl	Add a xylose residue to the glycan structure diagram.
	Fuc	Add a fucose residue to the glycan structure diagram.
	Gal	Add a galactose residue to the glycan structure diagram.
	Glc	Add a glucose residue to the glycan structure diagram.
	Man	Add a mannose residue to the glycan structure diagram.
	GalNAc	Add an N- Acetylgalactosamine residue to the glycan structure diagram.
	GlcNAc	Add an N-Acetylglucosamine residue to the glycan structure diagram.
	NeuAc	Add an N-acetylneuraminic acid residue to the glycan structure diagram.
	NeuGc	Add an N- glycolylneuraminic acid residue to the glycan structure diagram.
	Mass options of selected structure	Define derivitization, reducing end, and adduct ions.
	Delete	Delete selected residue.
	Delete All	Delete selected glycan structure.
	Linkage anomeric state	Defines anomeric state of the glycosidic linkage: select ?, α or β.
	Anomeric carbon	Defines anomeric carbon: select ?, 1, 2, or 3.
	Set linkage position	Defines position of the glycosidic linkage: select 1–6 or ?

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Button	Name	Action
	dRib	Add a *d*-ribose residue to the glycan structure diagram.
	View Menu	Show additional menu options.

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Table 8-4 Glycan Structure Editor shortcut menu commands

	Command	Description
	Structure Information	Display structure information.
	Save structure in database	Open the Add Glycan to Database dialog.
	Mass options	Define derivitization, reducing end, and adduct ions.
	Copy Glyco-CT/Glyde II string to clipboard	Copy respective XML description of the selected structure to the Clipboard.
	Copy image to clipboard	Copy an image of the selected structure to the Clipboard.
	Add glycan from Glyco-CT/Glyde II string	Open the **Import Glyco-CT/Glyde II String** dialog for entering structure as the respective XML description.
Residues		Opens a sub-menu with the following commands
	Add terminal	Select type (Antigen, Lactosamine or Lewis motif), subtype and linkage
	Add residue	Select type (Pentose, Hexose, Hexosamine, Acidic sugar, Heptose or Modification) and sub-type.
	Add structure	Select type (N-glycans, O-glycans, Glycosphingolipids, GAGs or Milk sugars) and sub-type.
	Add bracket	Add a bracket to the glycan structure diagram.
	Add repeating unit	Add a repeating unit to the glycan structure diagram.
	Residue properties	Display residue properties.
	Move residue counter-clockwise	Move selected residue counter-clockwise.
	Move residue clockwise	Move selected residue clockwise.

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8.6.4.6 Glycan Fragment Summary View

The Glycan Fragment Summary View displays a summary of the fragments detected within the selected glycan entry in the Glycan table and can be used to compare two or more glycans in the Glycan table. The calculated and measured *m/z*, intensity, and CFG structure of the detected fragments are displayed in ascending mass order.

Select and right-click the glycans to be compared and select **Show > Glycan Fragment Summary**. The side-by-side display enables the presence and composition of the fragment assigned to each peak list entry to be checked and compared for each glycan.

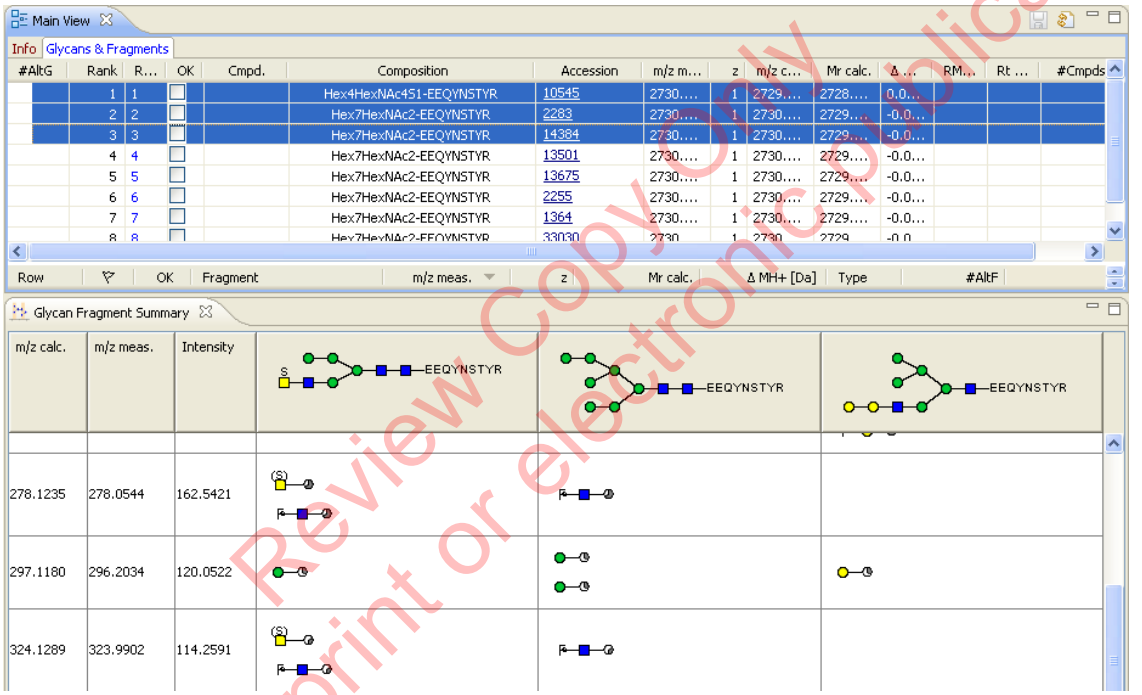

Figure 8-23 Comparison of three glycans using the Glycan Fragment Summary View

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9 Database Mining using Protein and Glycan Queries

A Query can be used to extract information from the ProteinScape database. Queries can be performed on any **Project Navigator** tree node by right-clicking the node and selecting the **Queries > New Protein/Glycan Query** option from the shortcut menu.

Alternatively, the Query View is opened through the main menu (**Window > Show View > Protein/Glycan Query**). Clicking **Add from Project Navigator** adds the currently selected **Project Navigator** tree node to the **Selected Items** field.

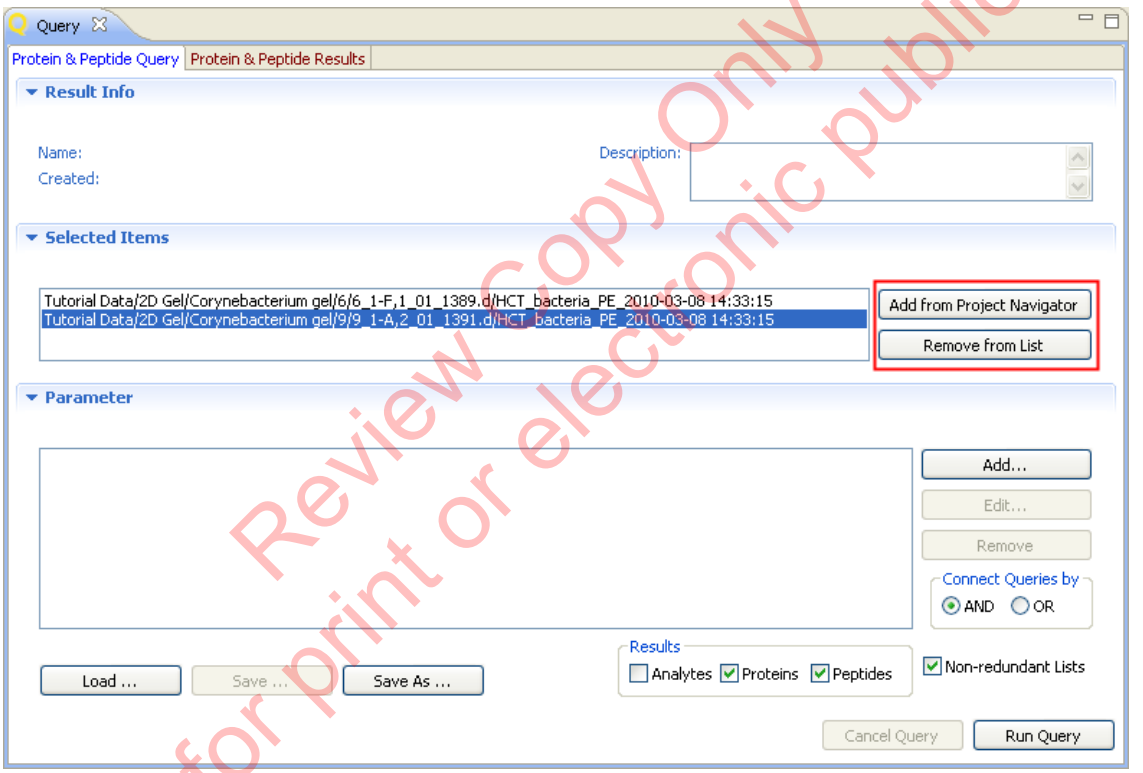

Figure 9-1 Protein Query View Protein & Peptide Query page

Additional nodes can be added to the scope of a Query by highlighting them in the **Project Navigator** tree and clicking **Add from Project Navigator**.

To remove nodes, highlight them in the **Selected Items** and click **Remove from List**.

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9.1 Defining Query Parameters

The parameters of a Query are listed in the **Parameter** area of the Query View.

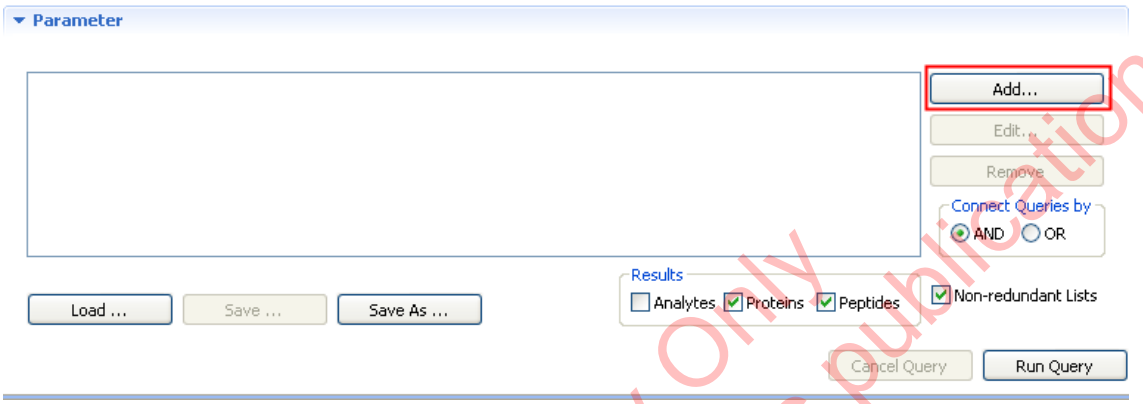

Figure 9-2 Query Parameter area of the Query View

Clicking **Add** launches the **Add Parameter** dialog box.

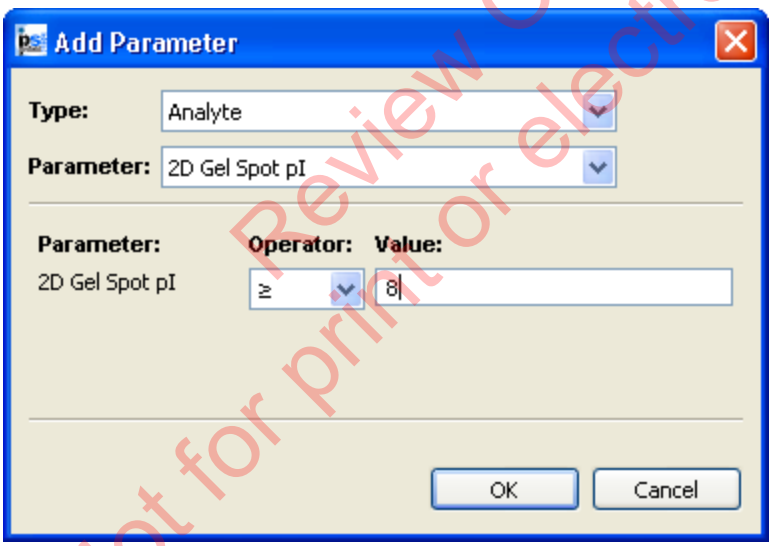

Figure 9-3 Add Parameter dialog

Define the desired parameter using the **Type** and **Parameter** drop-down lists.

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Choosing a **Parameter** opens a context-specific selection field where the **Parameter** can be defined using Operator (**=**, **≠**, **>**, **≥**, **≤** for numerical parameters; **contains**, **not containing** for text strings) and/or **Value** fields.

Table 9-1 Operators in Query parameters

Operator	Description	Example
*	Wildcard, unlimited length	AB*DE → ABXDE, ABXXXXXXDE
?	Wildcard, single character	AB?DE → ABXDE, ABYDE
[X]	Any single character within the brackets	AB[XY]DE → ABXDE, ABYDE

Additional operators can be added to text string parameters.

The **Edit** and **Remove** buttons are used to change and delete parameters respectively.

Multiple queries can be connected by selecting the relevant the **Connect Queries by** option.

9.1.1 Saving Query Parameters

Once the desired items and parameters have been added to a protein Query, it can be saved by clicking **Save As**. Enter a **Name** and **Description** (optional) in the Save Query Parameter dialog box and click **OK**.

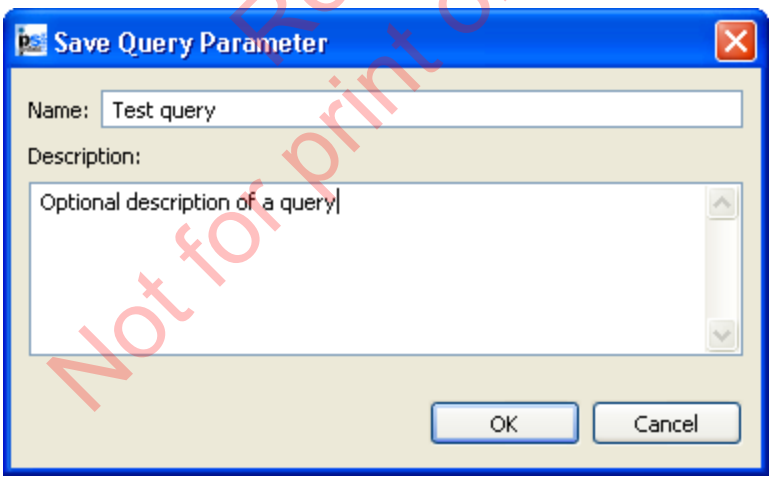

Figure 9-4 Save Query Parameter dialog

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9.1.2 Opening Existing Sets of Query Parameters

Existing protein queries stored on the ProteinScape server can be opened by clicking **Load** and selecting the desired query **Name** from the **Load Query Parameter** list.

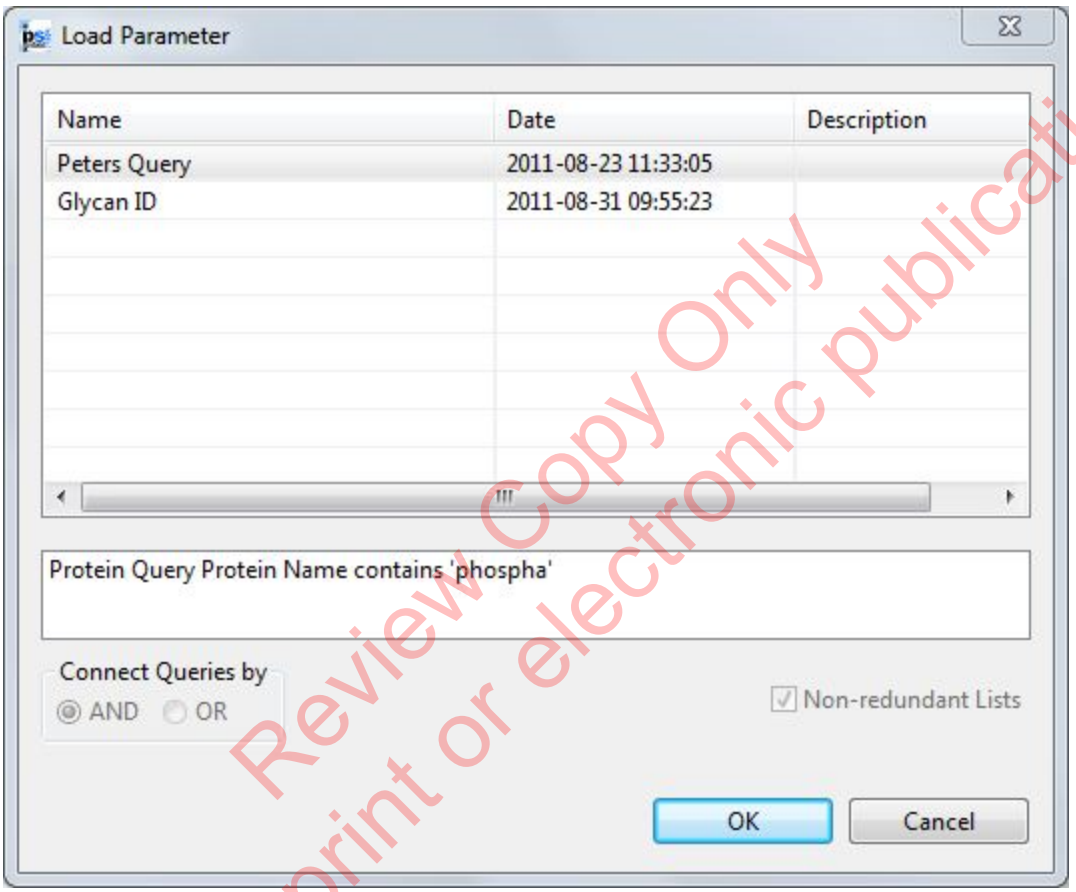

Figure 9-5 Load Query Parameter dialog

Add from Project Navigator and **Remove from List** are used to add or delete searched items in the **Selected Items** field.

The **Edit** and **Remove** buttons are used to change or delete **Query Parameters** respectively.

Changes to queries are saved by clicking **Save** and **OK**.

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9.1.3 Starting a Query

Select the desired result list(s) using the **Analytes**, **Proteins**, and **Peptides** check boxes in the **Results** field. Selecting **Non-redundant Lists** removes duplicate results from Query Result lists.

Click **Run Query** to start the query.

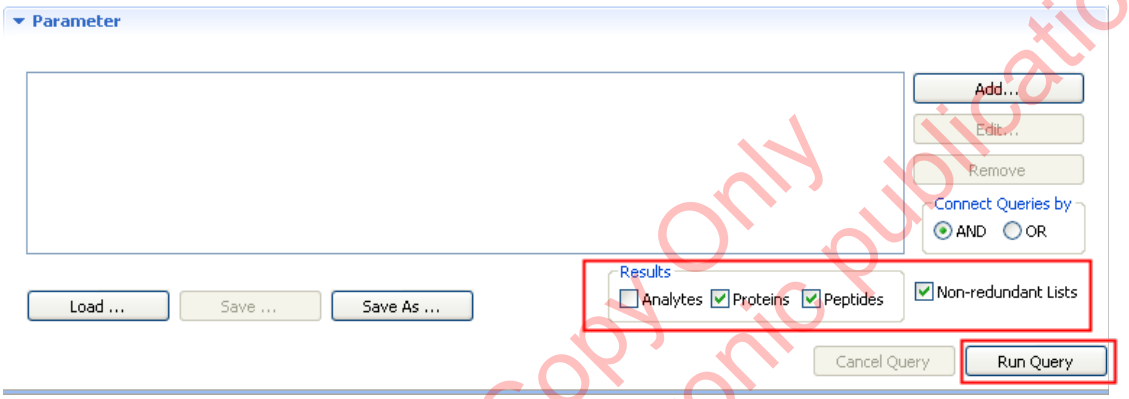

Figure 9-6 Clicking Run Query to start a Query

The **Executing Query** dialog box opens.

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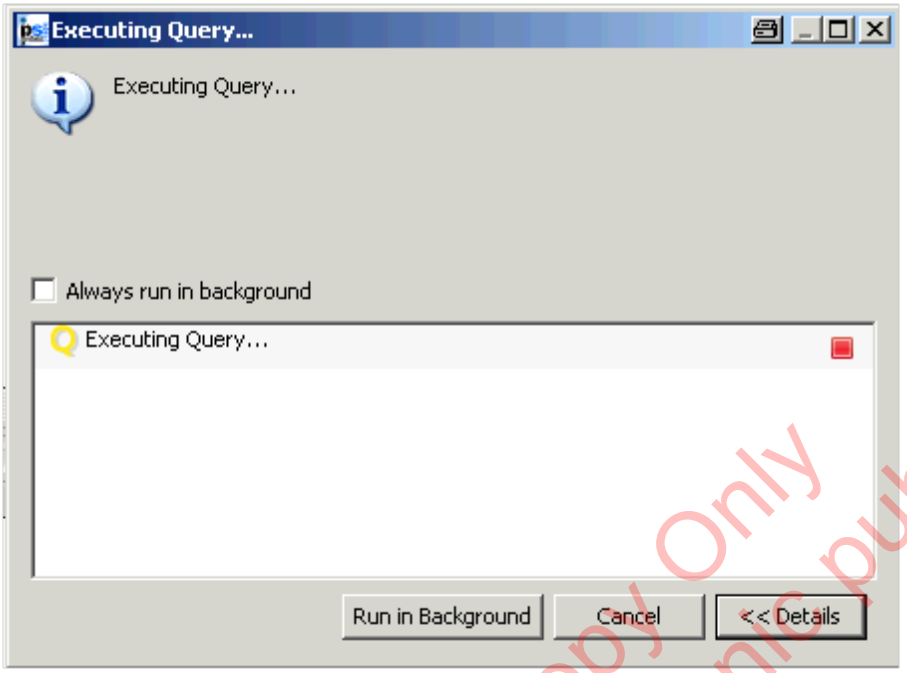

Figure 9-7 Executing Query dialog

Click **Run in Background** to minimize the dialog box for the current Query or check **Always run in background** to prevent the dialog box from appearing in the future.

Click **Details>>** to open a field showing the progress of the Query. Click **<<Details** to close the field.

Click **Cancel** to abort the Query.

9.1.4 Query Results

Query results are presented in tabular form on the **Protein & Peptide** / **Glycan & Fragment Results** page of the respective Query View. Selecting the relevant button in the Query View toolbar shows/hides the respective list.

To save a query result, select a destination node in the Project Navigator and click **Save Result**. Enter a **Name** for the result and click **OK**.

Note Query results are dynamic and will change if the underlying data are changed.

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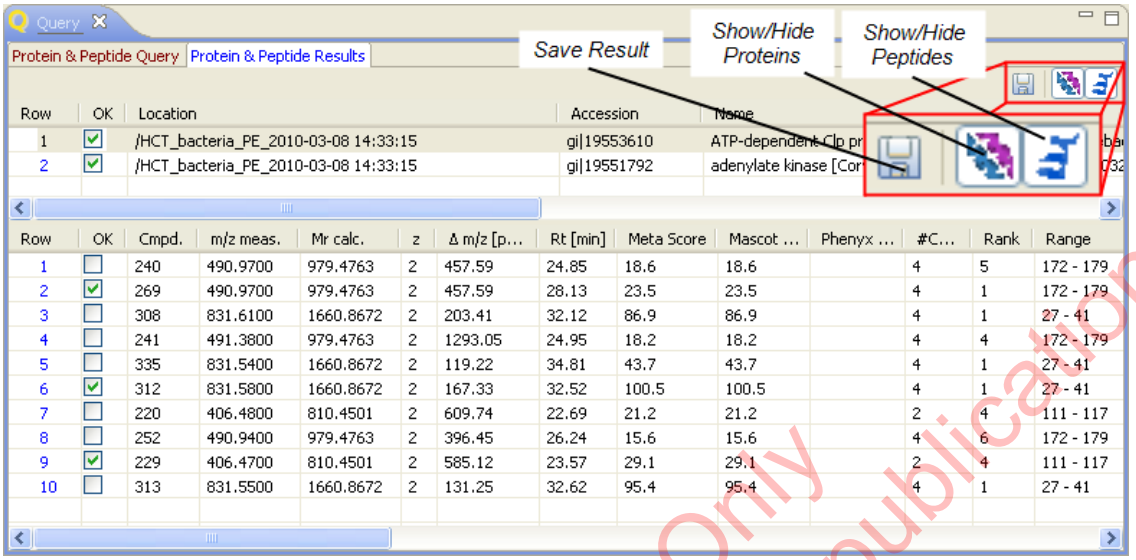

Figure 9-8 Protein Query View Protein & Peptide Results page

9.1.4.1 Query Results Table Shortcut Menu

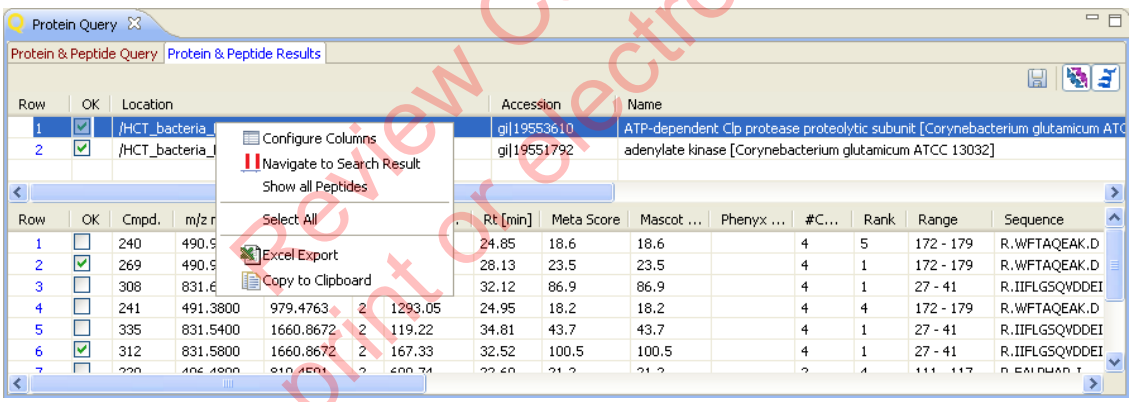
Protein Query
Protein & Peptide Query
Protein & Peptide Results
Row OK Location Accession Name
1 /HCT_bacteria_ gi|19553610 ATP-dependent Clp protease proteolytic subunit [Corynebacterium glutamicum ATC
2 /HCT_bacteria_ gi|19551792 adenylate kinase [Corynebacterium glutamicum ATCC 13032]
Configure Columns
Navigate to Search Result
Show all Peptides
Select All
Excel Export
Copy to Clipboard
Row OK Cmpd. m/z r Rt [min] Meta Score Mascot ... Phenyx ... #C... Rank Range Sequence
1 240 24.85 18.6 18.6 4 5 172 - 179 R.WFTAQEAK.D
2 269 28.13 23.5 23.5 4 1 172 - 179 R.WFTAQEAK.D
3 308 32.12 86.9 86.9 4 1 27 - 41 R.IIFLGSQVDDEI
4 241 491.3800 979.4763 2 1293.05 24.95 18.2 18.2 4 4 172 - 179 R.WFTAQEAK.D
5 335 831.5400 1660.8672 2 119.22 34.81 43.7 43.7 4 1 27 - 41 R.IIFLGSQVDDEI
6 312 831.5800 1660.8672 2 167.33 32.52 100.5 100.5 4 1 27 - 41 R.IIFLGSQVDDEI

Figure 9-9 Query Results table shortcut menu

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Table 9-2 Query Results table shortcut menu commands

Shortcut menu command	Action
Navigate to Search Result	The respective search result is highlighted in the Project Navigator and the Main View will display the respective **Info** or **Proteins & Peptides/Glycans & Fragments** tab.
Show all Peptides	Display all peptides in the peptide table, independent of the current protein selection.

9.2 Comparing Query Results

Comparative queries can be used to compare protein and peptide lists generated by simple queries. Between two and four saved **Query Results** can be compared.

Select two or more Query Result nodes in the **Project Navigator** tree, right-click and select **Compare Results** from the menu.

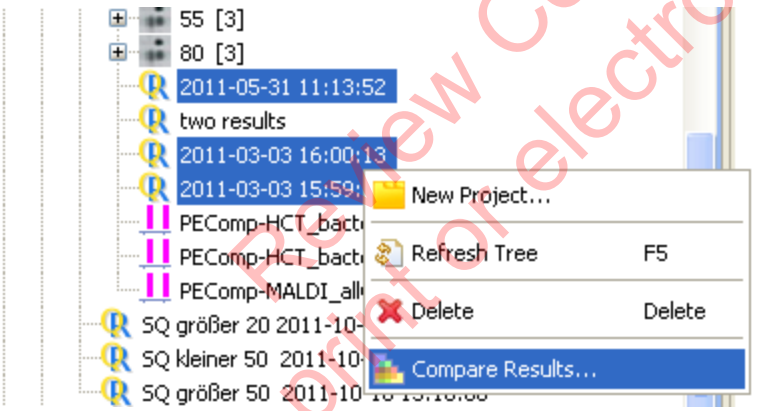

Figure 9-10 Starting a Query Results comparison

Users can also compare proteins and/or peptides present in protein and glycan search results. Comparisons are started by selecting and right-clicking two or more protein Search Results and selecting **Compare Search Results**. Results can be compared at the protein and/or peptide level. In this case, the complete Search Results will be compared without applying specific query parameters.

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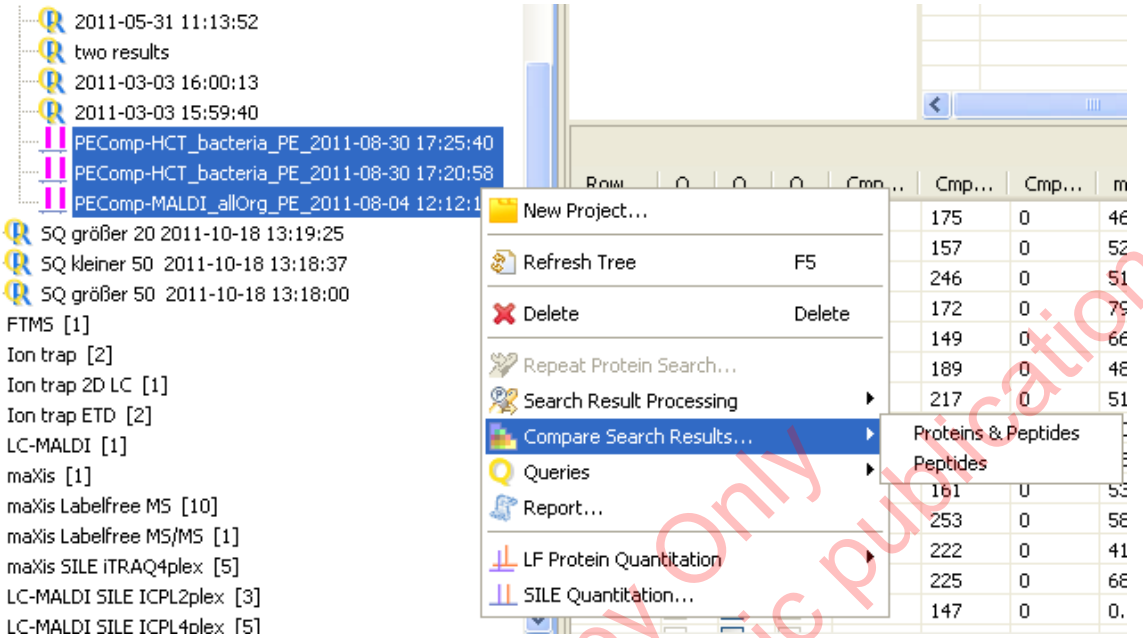

Figure 9-11 Starting a Search Results comparison

When comparing 2–4 query or search results, a Venn diagram displaying the relationship of the protein sets to one another is opened in the **Compare Query Results** View (see Figure 9-12).

Hovering the mouse pointer over a section of the diagram displays the subset (for instance AB) in the ToolTip. Clicking a set or intersection shown in the Venn diagram loads the respective proteins and peptides into the comparative tables. The location of the respective proteins and the peptides identified in the selected protein are also displayed.

Selecting **Navigate to Search Result** highlights the respective search result (A, B, C, …) in the Project Navigator. The Main View will display the respective **Info** or **Proteins & Peptides/Glycans & Fragments** tab.

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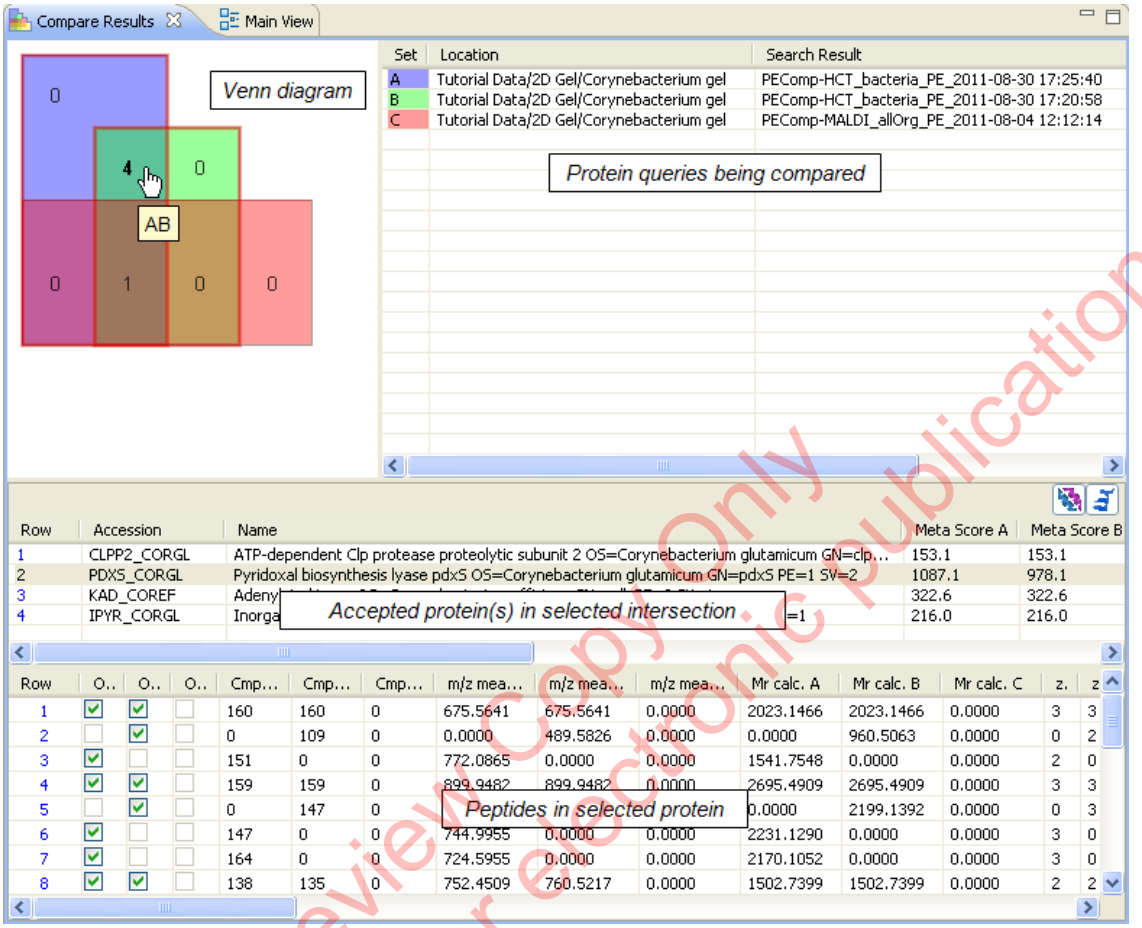

Figure 9-12 Compare Query Results View

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10 Reports

Five types of report can be created in ProteinScape:

- Protein Report
- Glycan Report
- Gel Report
- Spectrum Report
- Search Parameter Report

Reports can be generated using the buttons in the **Search Result Info** area of the protein or glycan Search Result Main View or right-clicking the respective node in the Project Navigator and selecting **Report**.

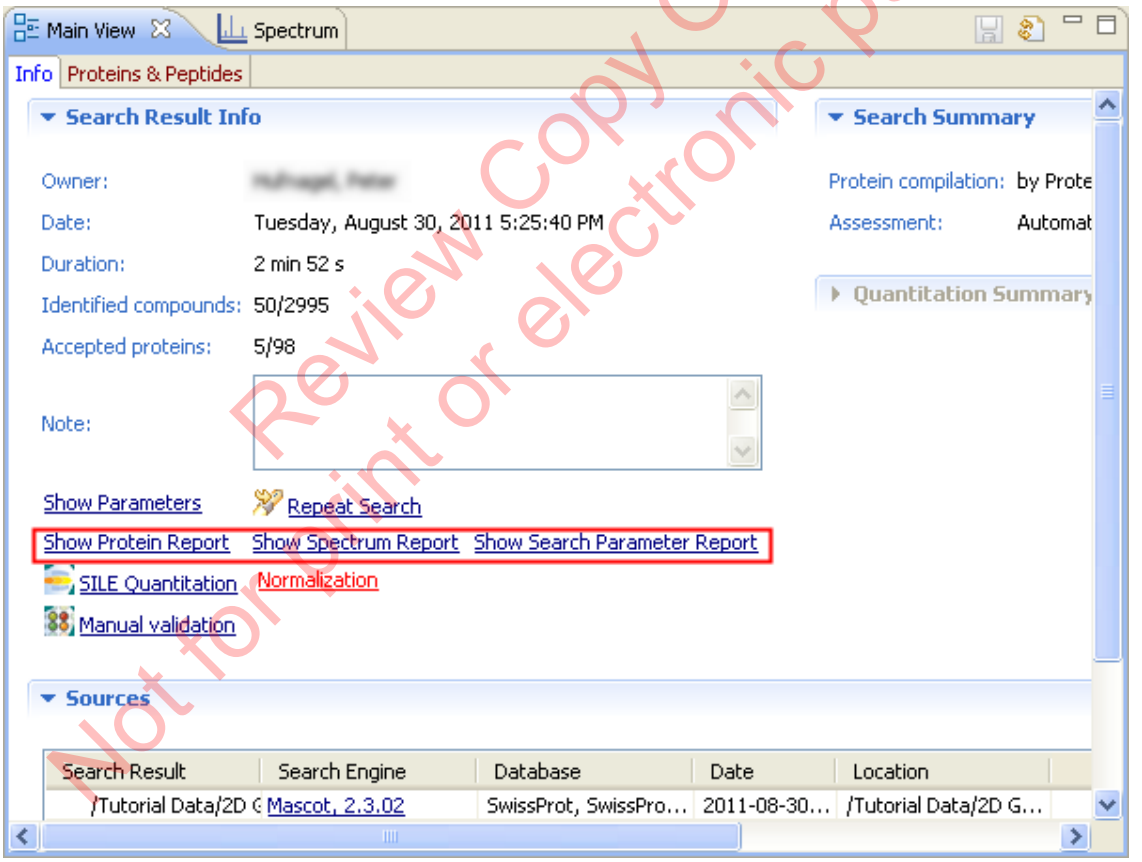

Figure 10-1 Protein search result report buttons

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Various output formats are available for each report (.html, .pdf, .doc, .xls, .ppt). After they are generated, html and pdf reports are displayed in the Report Viewer and can be printed or exported using the Report View shortcut menu. Microsoft Office–format reports can be opened or saved to disk.

10.1 Protein Report

The **Select Output Format** dialog offers the option to **Show Protein Sequences**. Selecting this option is not recommended if the report contains a large number of proteins.

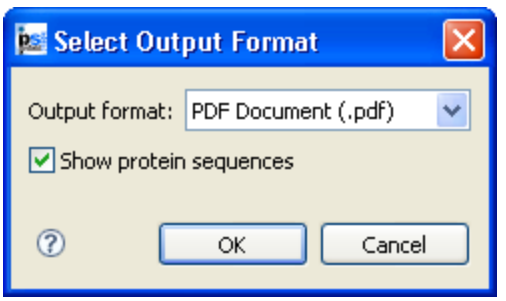

Figure 10-2 Protein Report Select Output Format dialog

The detailed report contains:

- Project information
- Search result information
- Protein information
- Protein sequence (optional)
- Compound list.

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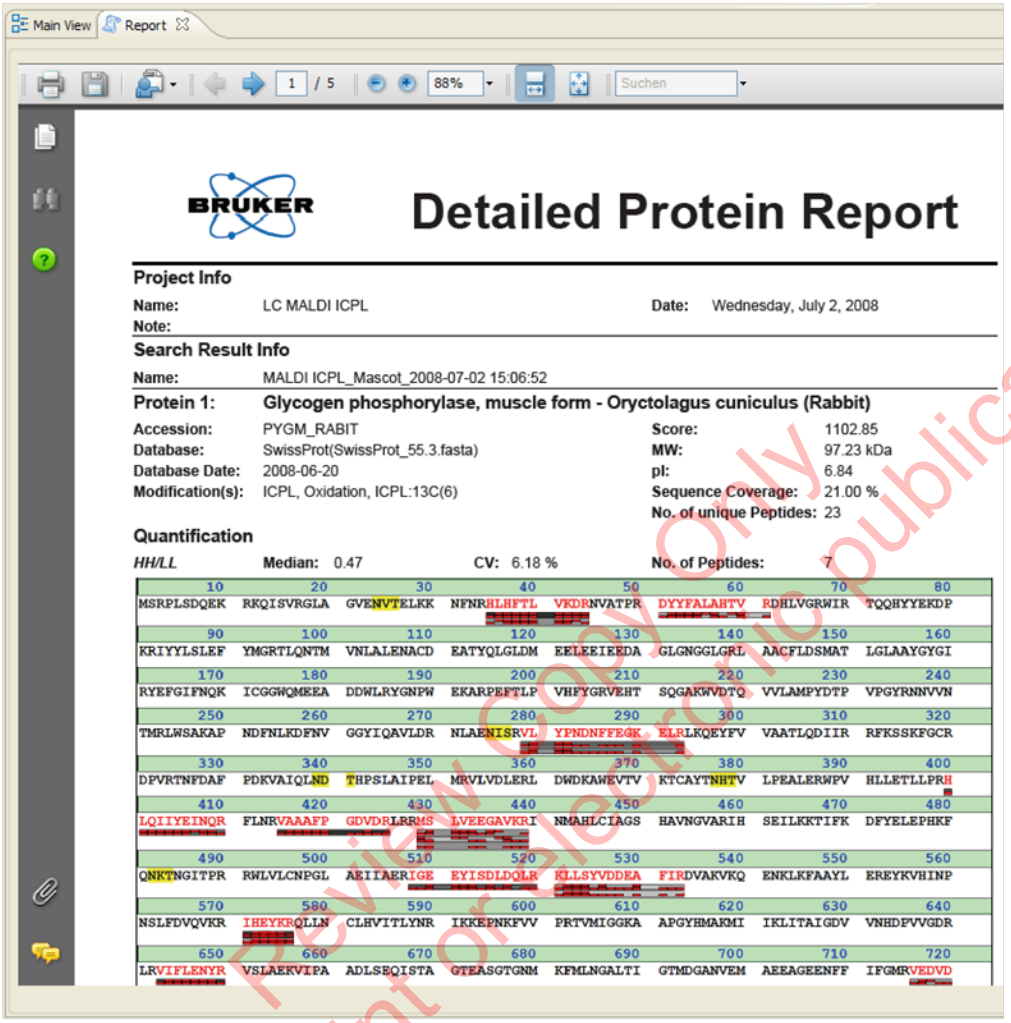
Main View
Report
1 / 5
88%
Suchen
BRUKER
Detailed Protein Report
Project Info
Name: LC MALDI ICPL
Date: Wednesday, July 2, 2008
Note:
Search Result Info
Name: MALDI ICPL_Mascot_2008-07-02 15:06:52
Protein 1: Glycogen phosphorylase, muscle form - Oryctolagus cuniculus (Rabbit)
Accession: PYGM_RABIT
Database: SwissProt(SwissProt_55.3.fasta)
Database Date: 2008-06-20
Modification(s): ICPL, Oxidation, ICPL:13C(6)
Score: 1102.85
MW: 97.23 kDa
pI: 6.84
Sequence Coverage: 21.00 %
No. of unique Peptides: 23
Quantification
HH/LL Median: 0.47 CV: 6.18 % No. of Peptides: 7
MSRPLSDQEK RKQISVRGLA GVENVTELKK NFNRHLHFTL VKDRNVATPR DYYFALAHTV RDHLVGRWIR TQQHYYEKDP
KRIYYLSLEF YMGRTLQNTM VNLALENACD EATYQLGLDM EELEEIEEDA GLGNGGLGRL AACFLDSMAT LGLAAYGYGI
RYEFGIFNQK ICGGWQMEEA DDWLRYGNPW EKARPEFTLP VHFYGRVEHT SQGAKWVDTQ VVLAMPYDTP VPGYRNNVVN
TMRLWSAKAP NDFNLKDFNV GGYIQAVLDR NLAENISRVL YPNDNFFEGK ELRLKQEYFV VAATLQDIIR RFKSSKFGCR
DPVRTNFDAF PDKVAIQLND THPSLAIPEL MRVLVDLERL DWDKAWEVTV KTCAYTNHTV LPEALERWPV HLLETLLPRH
LQIIYEINQR FLNRVAAAFP GDVDRLRRMS LVEEGAVKRI NMAHLCIAGS HAVNGVARIH SEILKKTIFK DFYELEPHKF
QNKTNGITPR RWLVLCNPGL AEIIAERIGE EYISDLDQLR KLLSYVDDEA FIRDVAKVKQ ENKLKFAAYL EREYKVHINP
NSLFDVQVKR IHEYKRQLLN CLHVITLYNR IKKEPNKFVV PRTVMIGGKA APGYHMAKMI IKLITAIGDV VNHDPVVGDR
LRVIFLENYR VSLAEKVIPA ADLSEQISTA GTEASGTGNM KFMLNGALTI GTMDGANVEM AEEAGEENFF IFGMRVEDVD

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Figure 10-3 Detailed protein report generated as a PDF document

10.2 Glycan Report

The **Select Output Format** dialog offers the option to **Show detailed glycan report**. Selecting this option is not recommended if the report contains a large number of glycans.

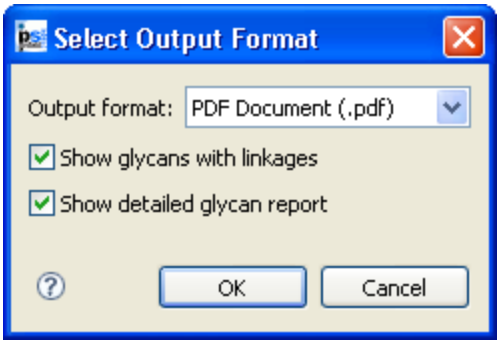

Figure 10-4 Glycan Report Select Output Format dialog

The detailed report contains:

- Project information
- Search result information
- Glycan structure
- Spectrum

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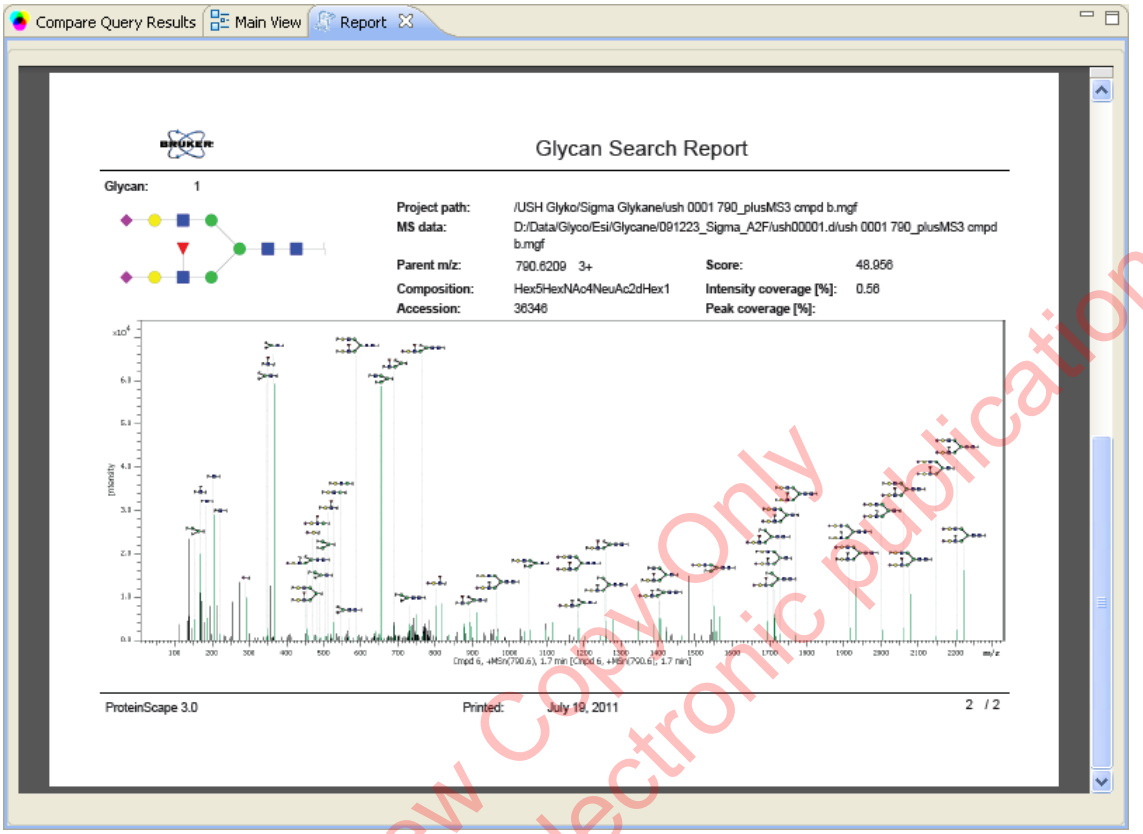

Figure 10-5 Detailed glycan report generated as a PDF document

10.3 Gel Report

Clicking the **Gel Report** link in the Main View of a 1D or 2D gel node generates a report containing project, sample, and protocol information; the gel image; and a list of spots/bands.

The **Select Output Format** dialog offers the option to **Show Detailed Protein Report**. Selecting this option includes sequence maps for all alternative proteins and is not recommended if a large number of alternative proteins are present.

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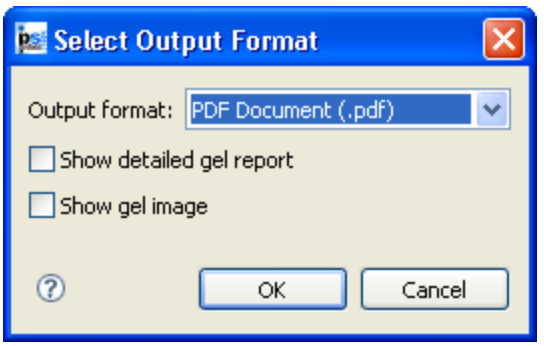

Figure 10-6 Gel Report Select Output Format dialog

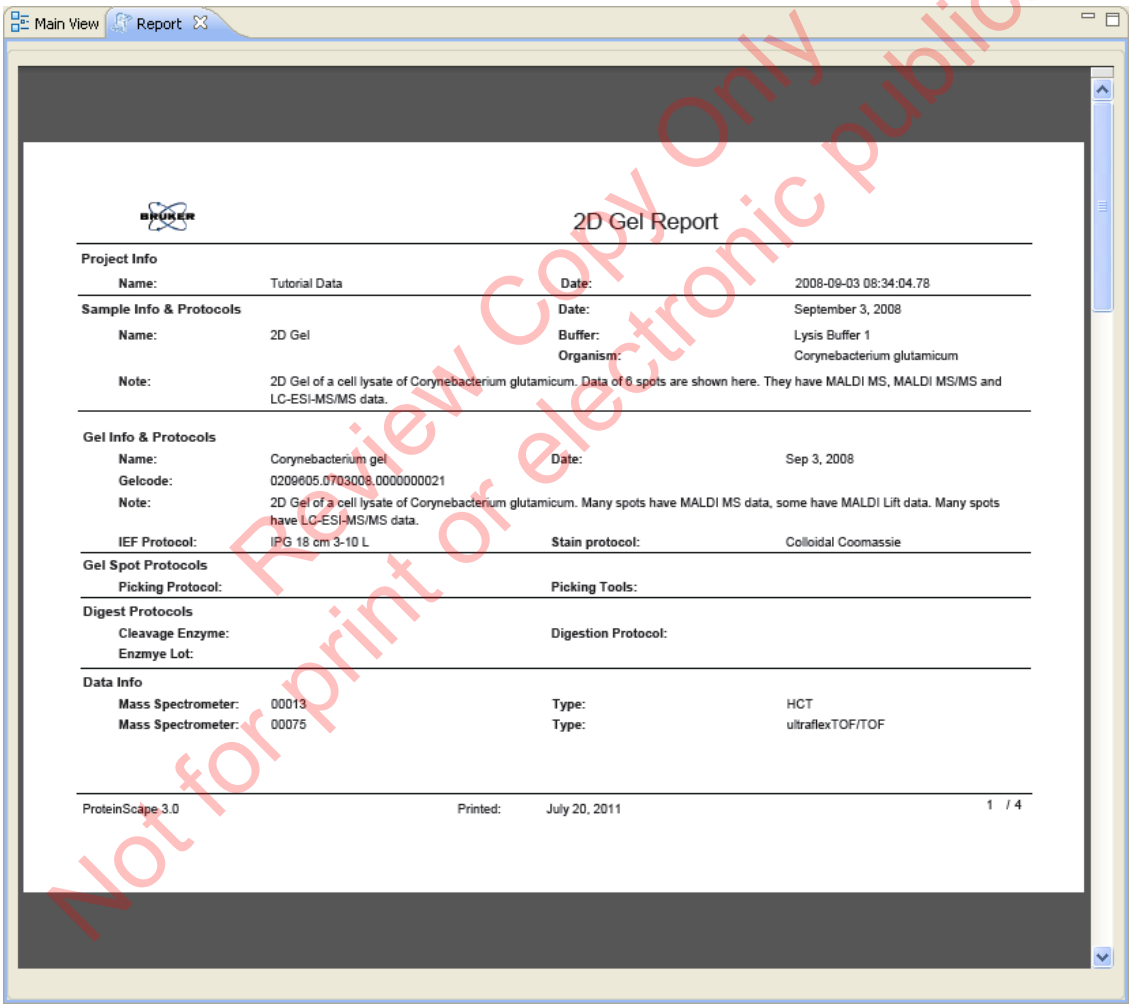
Main View
Report
2D Gel Report
Project Info
Name: Tutorial Data
Date: 2008-09-03 08:34:04.78
Sample Info & Protocols
Name: 2D Gel
Date: September 3, 2008
Buffer: Lysis Buffer 1
Organism: Corynebacterium glutamicum
Note: 2D Gel of a cell lysate of Corynebacterium glutamicum. Data of 6 spots are shown here. They have MALDI MS, MALDI MS/MS and LC-ESI-MS/MS data.
Gel Info & Protocols
Name: Corynebacterium gel
Date: Sep 3, 2008
Gelcode: 0209605.0703008.0000000021
Note: 2D Gel of a cell lysate of Corynebacterium glutamicum. Many spots have MALDI MS data, some have MALDI Lift data. Many spots have LC-ESI-MS/MS data.
IEF Protocol: IPG 18 cm 3-10 L
Stain protocol: Colloidal Coomassie
Gel Spot Protocols
Picking Protocol:
Picking Tools:
Digest Protocols
Cleavage Enzyme:
Enzmye Lot:
Digestion Protocol:
Data Info
Mass Spectrometer: 00013
Type: HCT
Mass Spectrometer: 00075
Type: ultraflexTOF/TOF
ProteinScape 3.0
Printed: July 20, 2011
1 / 4

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Figure 10-7 Gel report generated as a PDF document

10.4 Spectrum Report

Spectrum reports are generated by right-clicking within the axes of a Spectrum View and selecting **Show Report** from the menu.

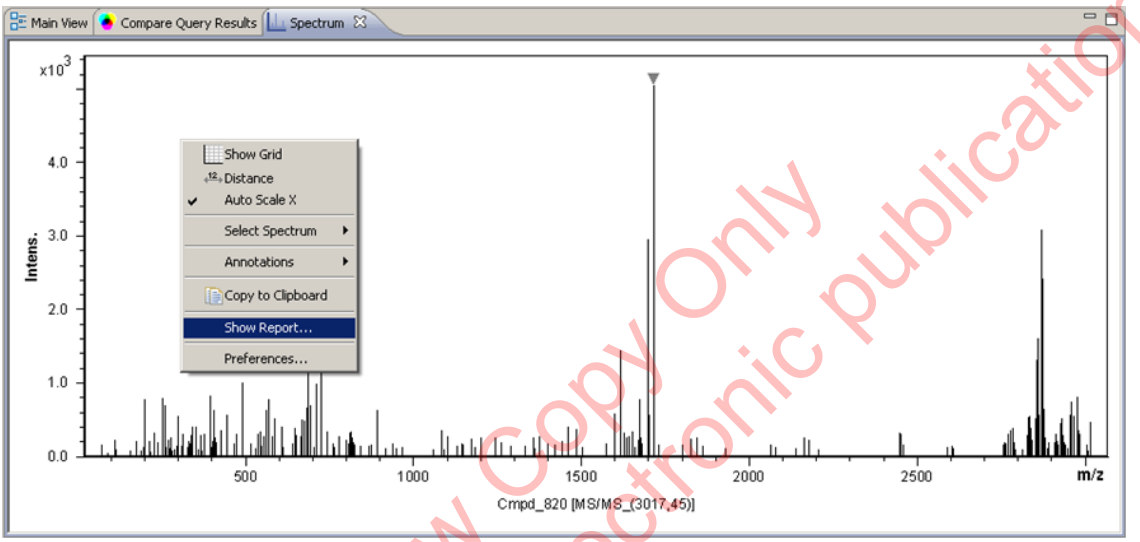

Figure 10-8 Generating a spectrum report through the Spectrum View shortcut menu

The Spectrum Report contains:

- Spectrum information
- Spectrum with annotations

10.4.1 Multiple Spectrum Report

The Multiple Spectrum Report is generated using the **Show Spectrum Report** button in the Search Result Info area of the **Search Result Main View** (see Figure 10-1).

Select the desired settings in the **Select Output Format** dialog box and click **OK**.

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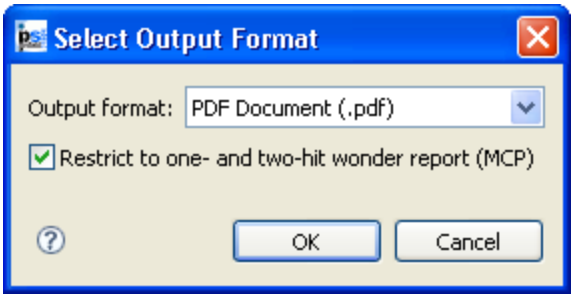

Figure 10-9 Spectrum Report Select Output Format dialog

The Spectrum Report contains:

- Spectrum information
- All spectra or only those spectra that contribute to the identification of proteins that are identified by one or two peptides (select **Restrict to one- and two-hit wonder report (MCP)**).

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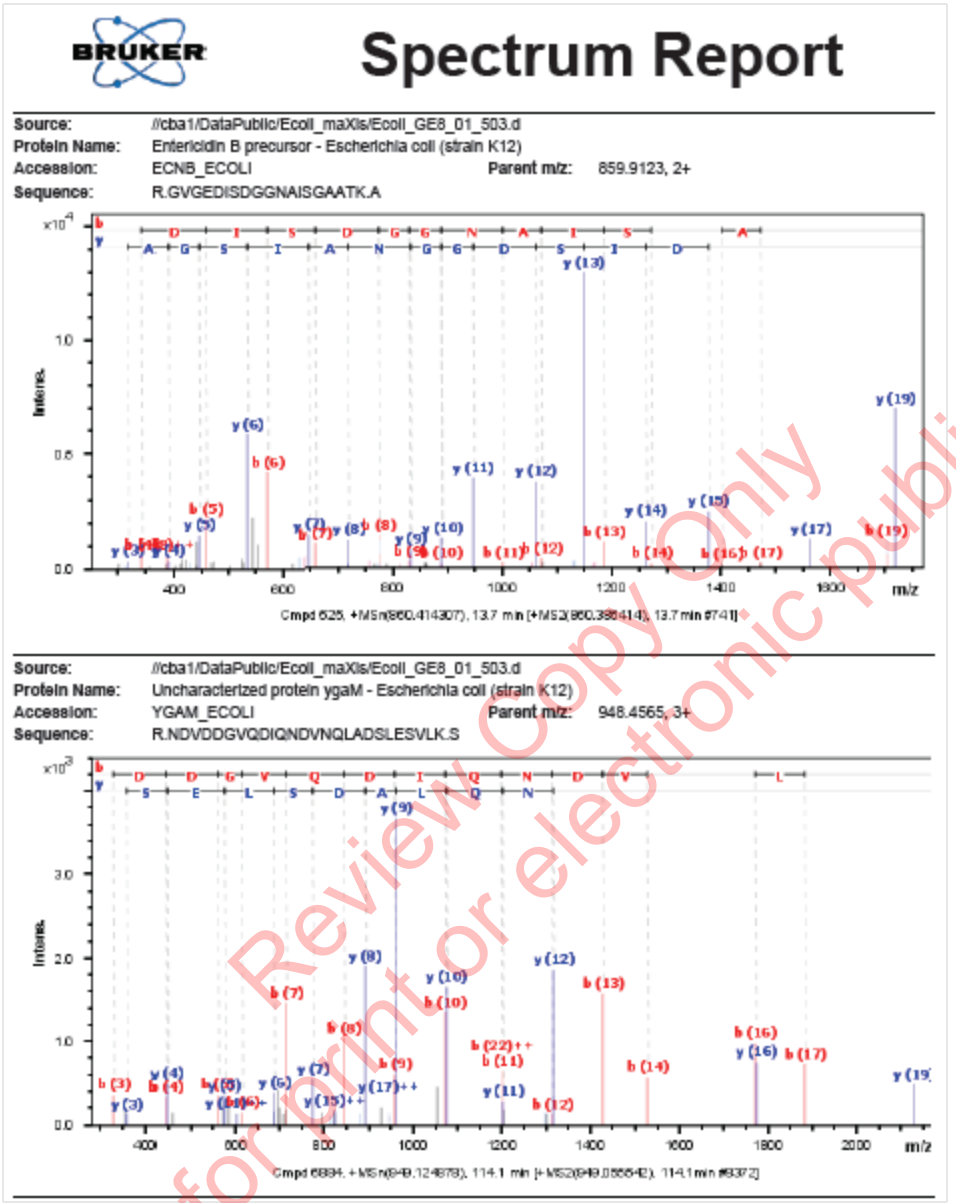

Figure 10-10 Spectrum report

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10.5 Search Parameter Report

Search Parameter reports are generated by clicking **Show Search Parameter Report** in the **Search Result Info** area of the **Info** page of the **Search Result Main View**.

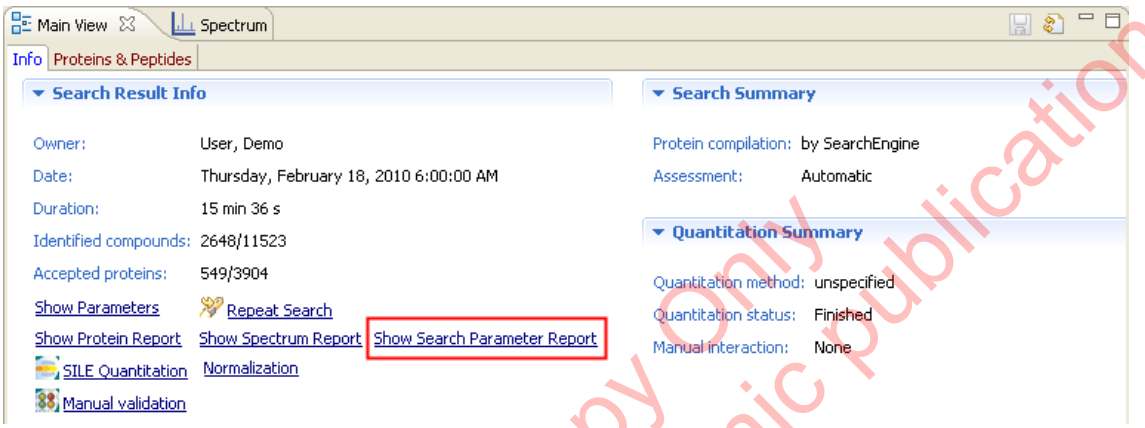

Figure 10-11 Generating a Search Parameter report

Select the desired format in the **Select Output Format** dialog and click **OK** to generate the report.

The report contains information on:

- Search method used
- Search engine used
- Protein list compilation
- Protein list assessment.

10.6 Excel Export

Tabular Views can be exported directly to Excel using the **Excel Export** button in the in the toolbar of the Table or Query Result View.

Alternatively, tabular Views can be exported directly to Excel by right-clicking in the Protein & Peptide table or **Processing** View and selecting the **Excel Export** option from the shortcut menu.

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Note When using this direct export functionality, all numbers are treated as text. This means that calculations or sorting in Excel may be problematic.

To convert cell contents to numbers, right-click highlighted cells, select **Format Cells** from the menu and select **Number** from the **Category** list.

Alternatively, use the **Copy to Clipboard** function (see section 10.7) to paste table contents into Excel. Using this function, numerical values are exported as numbers.

10.7 Copy to Clipboard

The **Copy to Clipboard** functionality is available in the toolbar of:

- Sequence View
- Query Result View

and the shortcut menu of all tables. The clipboard contents can be pasted into Microsoft Office, Open Office and related programs.

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Appendix A — Project Navigator Node Metadata Tables

This is the information added when new nodes are created in the navigator tree. Parameters marked with an asterisk are obligatory.

Node	Parameter entry fields on first page	Parameter entry fields on subsequent metadata pages
Project	Name*, Note	
Sample	Name*, Fraction, Volume, Concentration, Treatment, Note	Buffer, Cell Line, Concentration Protocol, Label, Organ, Organism, Pathology, Purification, Source, Subcellular Fraction
LC	Name*, Note	Column, LC Protocol, LC Technique, Solvents
Fraction	Name*, Number*, No. of successive fractions*, Volume, Note	Solvents
1D Gel	Name*, State, Note	Image file, Image name, Image note, Separation Protocol, Stain Protocol
Band	Name*, Number*, No. of successive bands*, Intensity, pI, MW, Note	Picking Protocol, Picking Tool
2D Gel	Name*, State, Note	Spot List File, Image File, Image Name, Image Note, IEF Protocol, PAA Protocol, Stain Protocol
Gel Spot	Name*, Number*, No. of successive spots*, Intensity, pI, MW, State, Note	Picking Protocol, Picking Tool
Digest	Name*, Note	Cleavage Enzyme, Digest Protocol, Enzyme Lot

** Obligatory field*

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A.1 Sample

Buffer	Name*, Version*, Note
Cell Line	Name*, Note
Concentration Protocol	Name*, Version*, Note
Label	Name*, Version*, Note
Organ	Name*, Note
Organism	Name*, Note, Org. Number, Org. Level, Taxonomy (ID), Database Search, System Name
Pathology	Name*, Note
Purification	Name*, Version*, Note
Source	Name*, Note, Contact Person's Fax, Contact Person's Phone, Contact Person's Email, Contact Person, Group Leader, Institution
Subcellular Fraction	Name*, Note

A.2 LC

Column	Name*, Note, Pore Size*, Diameter*, Length*, Date*, Brand*, Producer*
LC Protocol	Name*, Version*, Note, Temperature, Flow Rate
LC Tech-nique	Name*, Note
Solvents	Name*, Note
Fraction	
Solvents	Name*, Note

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A.3 1D Gel

Gel code	A specialized ID in the Proteineer Workflow comprising the Bruker robots Proteineer sp and Proteineer dp
State	Not Started, In Progress, Finished, Imported
Separation Protocol	Name*, Version*, Note
Stain Protocol	Name*, Version*, Note, Detail*, Agent*
Band	
Picking Protocol	Name*, Version*, Note
Picking Tool	Name*, Note, Radius*, Shape*

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A.4 2D Gel

Gel code	A specialized ID in the Proteineer Workflow comprising the Bruker robots Proteineer sp and Proteineer dp
State	Not Started, In Progress, Finished, Imported
IEF Protocol	Name*, Version*, Note, Voltage Hours*, Sample Loading*, Length (cm)*, Non Linear*, Stop*, Start*
PAA Protocol	Name*, Version*, Note, Dimension Z*, Dimension Y*, Dimension X*, Memo*, Concentration 2*, Concentration 1*, Gradient*, PercentCrossLink*
Stain Protocol	Name*, Version*, Note, Detail*, Agent*
Spot	
Area, Volume, coordinates	
State	Not Started, In Progress, Finished, Imported
Picking Protocol	Name*, Version*, Note
Picking Tool	Name*, Note, Radius*, Shape*

A.5 Digest

Cleavage Enzyme	Name*, Note, Does not cleave at, Is N-terminal*, Cleaves at*
Digestion Protocol	Name*, Version*, Note
Enzyme Lot	Name*, Note, Lot, Supplier*

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Appendix B — Main View Sections

Node	Info	Statistics	Protocols	Project Parameters	Project Access	Automated Searches
Project	X	X		X	X	
Sample	X	X	X	X		X
LC	X	X	X			
Fraction	X	X	X			
2D Gel	X	X	X			
Spot	X	X	X			
1D Gel	X	X	X			
Band	X	X	X			
Digest	X	X	X			
MS Data	X					
Search Result*	X					
Compounds	X					

** **Search Result Main View** also contains **Search Summary** and **Search Engine Summary***

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Appendix C — Protocol Parameters

This is the information added to nodes in the Protocol Navigator (see section 3.7).

Level	Parameter
Project	Project partners
Sample	Buffers
	Cell Lines
	Concentration
	Label
	Organ
	Organism
	Pathology
	Purification
	Source
	Subcellular Fraction
	WARP Method
1D Gel	Separation
	Stain
Band	Picking
	Picking Tool
2D Gel	IEF
	PAA
	Stain
Gel Spot	Picking
	Picking Tool
Digestion	Cleavage Enzyme
	Digestion

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Level	Parameter
	Enzyme Lot
LC	Column
	LC
	LC Technique
	Solvents
Fraction	Solvents
Pmf	MALDI Preparation
Fragment	MALDI Preparation
Search Method Mascot	Cleavage enzyme
	Default parent charge
	Mass spectrometer type
	Modification
	Sequence database
	Taxonomy
Search Method Phenyx	Cleavage enzyme
	Default parent charge
	Ion series
	Mass spectrometer type
	Modification
	Peptide mass tolerance unit
	Sequence database
	Taxonomy
	Turbo error tolerance unit

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